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SOME PHYSICAL PROPERTIES OF ENZYMES.*

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INTRODUCTION.

THE fact that enzyme solutions decrease in activity as the result of filtration has been frequently observed. Musculus[1] in 1874 called attention to the retention of the enzyme urase by filter paper, a fact which Lea[2] explained by the assumption that urase is not a soluble ferment. Miquel,[3] however, demonstrated the solubility of this enzyme.

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Wortmann[4] in 1890 attempted to demonstrate the presence of a ferment in green leaves. He tested the filtered aqueous extracts of crushed leaves for diastatic activity, and the leaves whose filtrates did not invert starch he declared free from diastase. This work elicited from Brown and Morris[5] (1893) the following:

> The absence of diastase from a clear filtrate obtained after merely digesting the crushed leaves with water for three or four hours is no sufficient proof that the leaf does not contain a starch-transforming enzyme; for it is well known to those who have spent much time in separating such enzymes from vegetable or animal tissues that the protoplasm often parts with its enzyme with difficulty, and that it is often almost impossible to obtain from such a preparation a clear filtrate which has any hydrolyzing action, although an energetic hydrolysis may be produced by contact with the tissue itself, or by the employment of a turbid filtrate containing the finely divided tissue in suspension.

They cite in evidence the following experiment:

> The leaf of Helianthus tuberosus was extracted by the prolonged action of water upon the crushed and pounded fresh leaf. The influence of micro-organisms was guarded against by the use of chloroform. The action of the filtered extract was then compared with a like maceration of the leaves themselves. The diastatic activity of 10 g. of the dried leaf of Helianthus, acting by contact, was found to be 3.78, while that of the filtered extract of 10 g. of the same leaf was 0.53.

They refer, further, to the observation of Brown and Herron[6] (1880) that the clear, filtered, aqueous infusion of tissue of the small intestine of the pig had but slight hydrolytic action on starch, whereas contact with the tissue itself produced pronounced hydrolysis.

Vines[7] (1891) found that the turbid extract of green leaves when merely strained was much more active than the clear extract obtained by filtration.

Kastle and Loevenhart,[8] in their work on lipase, macerated the pancreas with sand, then mixed the mass with water, and simply strained through linen or cotton cloth. They report:

> The effect of filtration was invariably to diminish the activity of the solution. This was proved experimentally for aqueous and glycerin extracts of both pancreas and liver. The same was also found to hold for the pancreatin of Parke, Davis & Co. A two per cent. solution of this preparation is yellowish in color and is turbid. In this shape it possesses weak but measurable lipolytic power. On filtering a great many times, a clear yellowish solution is obtained, which possesses only a trace of its original lipolytic activity.

To show further the effect of filtration on a liver extract, they cite the following:

> Before filtering, 1 c.c. of a 10 per cent. liver extract was found to hydrolyze 6.28 per cent. of ethyl butyrate in fifteen minutes at 40°. After filtering repeatedly through the same filter paper, it hydrolyzed only 2.76 per cent., showing a falling off in activity of over one-half as the result of filtration. The removal of lipase along with suspended matter in the solution was observed in still another connection. When an active pancreatic extract acts on ethyl butyrate at 40°, the butyric acid produced soon coagulates the proteids in the extract. On filtering it was found that the filtrate possessed little or no lipolytic power, whereas the proteid coagulum was found to be exceedingly active.

PAPER FILTRATION OF PTYALIN.

In order to determine whether or not ptyalin could be removed from saliva by filtration through paper, the following experiment was carried out:

Two hundred cubic centimeters of saliva were collected by mastication of paraffin, and subjected to filtration through eight double filters of Schleicher and Schüll's hardened filter paper, No. 575. These filters were arranged as follows: In each of two funnels were placed two folded filter papers. The funnels were arranged one above the other on a ring stand, so that the stem of the upper funnel touched the side of the filter paper in the lower one. The stem of the lower one passed into an Erlenmeyer flask. Thus the saliva poured into the upper funnel passed through four thicknesses of paper before reaching the flask. This filtrate, designated as "2 × 2," then passed through a second set of filters similarly arranged and gave filtrate "4 × 2," the passage of which through another set of double filters produced filtrate "6 × 2," which in turn filtered as before, through a fourth set of new double papers, gave as final filtrate, "8 × 2." In all of our paper-filtration experiments, the enzyme solution was filtered through eight double filters of hardened paper.

Previous to the filtration through the eight double filters, the saliva was filtered through one thickness of ordinary filter paper (S. & S., No. 595) to remove the mucin and solid particles which otherwise retarded the subsequent filtration.

In order to ascertain the decrease in diastatic activity, a definite quantity of each filtrate was added to a definite volume of a starch solution, and the resulting mixture was kept at a fixed temperature in a water bath. From time to time a portion of the liquid was removed and tested with iodine until the achromic point was reached.

A preliminary trial indicated the necessity of using but small portions of the successive filtrates in order to determine the progressive decrease in activity of the filtered solution, since in concentrated solutions the action of ptyalin is evidently not directly proportional to the amount of the enzyme present. The following experiment will make this clear:

Two hundred cubic centimeters of saliva, previously passed through one thickness of ordinary filter paper, were subjected, as above described, to eight successive double filtrations; each filtrate and a portion of unfiltered saliva which was set aside as control, were tested by adding 0.5 c.c. of each to 30 c.c. of a 1 per cent. solution of starch, and the mixture was allowed to digest at 34–37°. A similar set was made, differing only in that the control and each filtrate were tested by adding 0.1 c.c. of each to 30 c.c. of a 1 per cent. starch solution. The filtration of the first set consumed about two hours, while that of the second required three and one-quarter hours. The following table gives the results, indicating the number of minutes required in each test to reach the achromic point:

TABLE I.

	Digested with 0.5 c.c.	Digested with 0.1 c.c.
Control	10 min.	30 min.
"2 × 2" filtrate	10 min.	42 min.
"4 × 2" filtrate	10 min.	50 min.
"6 × 2" filtrate	8 min.	62 min.
"8 × 2" filtrate	6 min.	Pink in 82 min.

The above, then, indicates that, although ptyalin is removed from its solution by filtration, this fact is not made manifest unless the quantity of saliva employed in testing is small enough to prevent the masking of the loss by the activity of the remaining enzyme. For this reason in the following experiments diluted saliva was employed.

EXPERIMENT I.

Twenty cubic centimeters of saliva were collected by mastication of paraffin and filtered through one thickness of ordinary filter paper. This filtrate was diluted to 200 c.c. with distilled water. Filtration was then carried out as above described. A portion of the original diluted saliva was set aside (10 per cent. solution) in a test-tube at the same temperature and light conditions as obtained for the filtering saliva. One cubic centimeter of this control solution was added to 30 c.c. of the starch solution at the same time that 1 c.c. of each successive filtrate was similarly treated. A filtered 1 per cent. starch solution containing glycerin was employed for these tests. The filtering liquid and the control were protected from light by black cloth. The progress of digestion in each case was noted by removing from each tube from time to time 2 c.c. of the solution and testing this with a dilute iodine solution. The pipette used was rinsed thoroughly each time with boiling distilled water.

The time required to reach the achromic point is indicated in the following table:

TABLE II.

1 c.c. 10 per cent. Saliva + 30 c.c. of Starch Solution, Digested at 34°.

Control	Achromic Point	Filtrate from	Achromic Point
“2 × 2”	20 min.	“2 × 2”	20 min.
“4 × 2”	20 min.	“4 × 2”	20 min.
“6 × 2”	20 min.	“6 × 2”	25 min.
“8 × 2”	20 min.	“8 × 2”	Deep purple in 1 hr. 45 min.

The time consumed in filtration was two hours and ten minutes.

TABLE III.

0.5 c.c. Saliva + 30 c.c. of Starch Solution, Digested at 34°.

(This experiment is like the preceding save that 0.5 c.c. of control and of each filtrate was added to 30 c.c. of the starch solution.)

Control	Achromic Point	Filtrate from	Achromic Point
“2 × 2”	40 min.	“2 × 2”	48 min.
“4 × 2”	40 min.	“4 × 2”	54 min.
“6 × 2”	36 min.	“6 × 2”	70 min.
“8 × 2”	36 min.	“8 × 2”	Blue in 1 hr. 30 min. Purple in 18 hrs.

The duration of the filtration process in this case was two hours and thirty minutes.

Further experiments gave the same results as the preceding, showing without a doubt that saliva is relieved of its ptyalin by filtration through hardened filter paper.

To what, then, is this result due? Three answers present themselves: the molecules of ptyalin, as in the case of a precipitate, may be mechanically retained by the filter; or the aeration or the surface action, if any, may have a destructive effect; or, finally, the ptyalin may have entered into firm combination with the material of the filter paper, *i. e.*, it may have been *fixed*.

EXPERIMENT III. (BERKEFELD FILTRATION.)

To determine whether the paper acted by mechanical retention, the following experiment was carried out:

A.—20 c.c. of saliva, filtered through a single ordinary filter paper, were diluted to 200 c.c. with distilled water. The entire quantity, less 5 c.c. which were set aside as control, was filtered through a No. 5 Berkefeld filter (1 × 8 inches) at room temperature. The diastatic action of the filtrate was tested and compared with that of the control solution.

B.—Same as A, save that filter was warmed to 50° by repeated washings with distilled water warmed to 50° and 195 c.c. of 10 per cent. saliva warmed to 50° filtered through bougie. Control of unfiltered saliva warmed to 50°. Tested filtrates and controls as before. The time required to produce achromicity is indicated in the table below:

TABLE IV.

05 c.c of Saliva+30 c.c. Starch Solution, at 34°.

	Temperature	Filtration Pressure	Rate of Filtration	Achr. Pt. of Filtrate	Achr. Pt. of Control
A.......	Room	740 mm.	7.5 min. per 100 c.c. *ca.*	40 min.	40 min.
B.......	50°	720 mm.	7 min. per 100 c.c. *ca.*	40 min.	40 min.

This disposes of the idea of the mechanical retention of the molecules. It is quite inconceivable that the molecules should be retained by the comparatively large pores of the filter paper, and be allowed unimpeded passage through the pores of the Berkefeld filter which denies passage to minute bacteria. Some factor other than mechanical retention has been at work.

EXPERIMENT IV.

To determine the effect of aeration the following experiment was performed:

Over the narrowed end of a piece of amber glass tubing 46 cm. long and 1½ cm. wide was slipped a piece of rubber tubing 5 cm. long. This tube was provided with a capillary glass tip and with a Hofmann clamp. The amber glass tube was then filled to a depth of about 35 cm. with clean glass beads, which were prevented from escaping from the tube by a thin plug of glass wool. The tube was then clamped in an upright position to a ring stand. The Hofmann clamp was closed, and about 25 c.c. of 10 per cent. saliva (filtered through ordinary filter paper before dilution) were poured into the tube. A rubber stopper carrying an L-shaped glass tube closed the upper end of the tube, and this L was connected by rubber tubing with an aspirating pump. Suction was started and the Hofmann clamp was opened sufficiently to allow a vigorous, but not too violent, aeration of the contents of the tube. This aeration was allowed to continue for twenty-four hours, the amylolytic action of the liquid being tested before aeration was begun, also at the end of three, six, nine, and twenty-four hours respectively. A control of unaerated 10 per cent. saliva set aside at room temperature under a black cloth was likewise tested at the same intervals. Saliva, control and aerated, was tested by adding 0.5 c.c. to 30 c.c. of 1 per cent. starch solution, and digestion was allowed to proceed at 35°. The time required to produce achromicity is indicated in the following table:

TABLE V.

Control	Achromic Point	Saliva Aerated for	Achromic Point
Original	32 min.	....	....
3 hrs.	20 min.	3 hrs.	24 min.
6 hrs.	22 min.	6 hrs.	24 min.
9 hrs.	38 min.	9 hrs.	38 min.
24 hrs.	24 min.	24 hrs.	24 min.

The slowness of digestion in the nine-hour set was probably due to the fact that the digestion temperature was 32°, whereas that of the other sets was 35°.

It is evident from the above that aeration, even for twenty-four hours, is without appreciable injurious effect on a 10 per cent. saliva.

Szumowski[9] has demonstrated the fixation of enzymes (ptyalin, rennin, pepsin, trypsin) by fibrin, and Effront[10] states that enzymes are fixed by different substances, such as silk and fibrin. Chevreul[11] (1853) tested the fixing or mordant action of wool, cotton, and silk on such substances as sodium chloride, sulphuric acid, mercuric chloride, hydrochloric acid, lime water, baryta water, alum, barium nitrate, lead nitrate, and ferrocyanide of potassium. To quote from one experiment:

> Thus cotton absorbs $HgCl_2$ and water in the proportion of the solution, and moreover, it retains a portion of it with so much force that, after having been washed until the wash-water no longer precipitates $AgNO_3$, it is colored by a solution of H_2S and it acts otherwise than pure cotton on the coloring principles of cochineal, logwood, and madder.

"The action of solids in altering solutions," says Chevreul, "shows how filters can act in a chemical manner on the liquids which traverse them."

Musculus (*loc. cit.*) shows that if urine in active alkaline fermentation be filtered through paper, this paper, after being washed until the wash-water is free from any alkaline reaction and dried at 35 or 40°, retains, even for months, a something capable of exciting alkaline fermentation of urea. This something he considered to be "torulæ," retained in the pores of the paper. It is possible, in view of the demonstration by Miquel (*loc. cit.*) of the solubility of urase, that the filtered paper had fixed the urase.

O'Shea[33] found that lead was retained by various filter papers (English, French, and German make) on filtering lead salts. An experiment shows that a S. & S. filter, 589, absorbed from 50 c.c. of lead acetate 0.12 mg. of lead, 0.02 mg. of which was removed by a first and none by a second washing. On refiltering 0.24 mg. was absorbed, none of which was removed by subsequent washing.

That hardened filter paper fixes ptyalin is proved by the following experiments:

EXPERIMENT V.

In the aeration tube above described were placed about 1,500 sq. cm. of hardened filter paper, cut into bits about ½ cm. sq. and then crumpled. The papers in the tubes were first washed with distilled water and then with 10 per cent. saliva (filtered through ordinary filter paper before dilution), after which 35-40 c.c. of the 10 per cent. solution were added and aerated over the paper bits, the same as before over beads, and the diastatic activity was tested at intervals of three, six, nine, and twenty-four hours. A portion of unaerated saliva was set aside at room temperature under a black cloth for control and was tested at the same intervals.

The time required to produce achromicity is shown in the following table:

TABLE VI.

0.5 c.c. 10 per cent. Saliva + 30 c.c. 1 per cent. Starch Solution at 35°.

Control	Achromic Point	Saliva Aerated for	Achromic Point
Original..........	36 min.	....	....
3 hrs............	36 min.	3 hrs............	64 min.
6 hrs............	32 min.	6 hrs............	60 min.
9 hrs............	32 min.	9 hrs............	56 min.
24 hrs............	32 min.	24 hrs...........	60 min.

Although aeration over beads produced no change in activity of saliva, aeration over paper did. The saliva was now drained off, and the papers were washed by rinsing and aerating with distilled water repeatedly, until the wash-water no longer exhibited a trace of ptyalin.

A number of shreds of paper were then removed from the tube, rinsed in a beaker of water, and added to 30 c.c. of a 0.1 per cent. starch solution. To another tube containing 30 c.c. of the starch solution was added an equal quantity of fresh crumpled bits of hardened filter paper, washed in distilled water. This latter served as control. Both were placed in a thermostat at 35°. Portions of contents of each tube responded to iodine with blue coloration at the end of an hour. Absence from laboratory prevented further test until at the end of forty hours, at which time the *starch in the tube* *which contained paper aerated with saliva gave achromic reaction with iodine,* while starch in control tube showed no inversion whatever.

At this time the water was drawn off from the washed papers which remained in the tube, and glycerin was added. Aeration was then proceeded with for thirty minutes, at the end of which time the glycerin was withdrawn and 10 c.c. added to 10 c.c. of the 0.1 per cent. starch solution. This was placed in water bath at 35°, and portions were tested with iodine at intervals indicated below, with the following results:

At the end of fifteen minutes the color produced by the iodine was blue. When next tested at the end of twenty-five minutes, no color reaction was obtained. This shows that, as will be borne out in subsequent experiments, the glycerin had extracted the ptyalin which had been retained by the filter paper.

EXPERIMENT VI.

The saliva was aerated over paper as in the preceding experiment. The aerated saliva and control were tested at the end of three hours and fifteen and one-half hours.

TABLE VII.

0.5 C.C. OF 10 PER CENT. SALIVA + 30 C.C. OF 1 PER CENT. STARCH SOLUTION AT 35.5°.

Control	Achromic Point	Saliva Aerated for	Achromic Point
3 hrs............	20 min.	3 hrs............	20 min.
15½ hrs............	20 min.	15½ hrs..........	20 min.

In the preliminary filtration experiments it was seen that, although the liquid experimented with loses some of its ptyalin, the activity of the remaining ptyalin hides this fact. That this is also the case with saliva aerated in Experiment VI is shown by the following:

The paper bits in the tube were washed with distilled water until all traces of free ptyalin were removed, and the paper was then taken from the tube. A number of the bits were added to 30 c.c. of a 0.1 per cent. starch solution and placed in the thermostat at 36°. A corresponding number of fresh bits were likewise added to 30 c.c. of starch solution for control. The experiment was further controlled by placing in the thermostat a third tube containing 15 c.c. of the last wash-water, added to 15 c.c. of the starch solution. Portions of the liquid in each tube were tested from time to time with iodine, with the following results:

Control wash-water + starch solution — blue in 2½ hrs.
Control paper + starch solution — blue in 2½ hrs.
Paper aerated with saliva + starch solution — achromic in 2½ hrs.

At the end of nineteen hours both controls gave purple reaction with iodine.

EXPERIMENT VII.

The bits of paper from Experiment VI not employed in making the above test were kept in refrigerator for twenty-one hours. They were then returned to the washed tube, which was labeled *A*. In another similar tube, labeled

B, was placed an approximately equal amount (about 1,000 sq. cm.) of paper in crumpled bits. To each tube was added 70 c.c. of 10 per cent. saliva, filtered before dilution through ordinary filter paper. The contents of the tubes were then aerated side by side at equal rates of aeration for twenty-four hours, and both were controlled by an unaerated 10 per cent. saliva kept at room temperature under a black cloth. The control was tested at the same intervals as the aerating saliva. The saliva was extremely active, and for that reason in the following tests only 0.1 c.c. of saliva was employed.

TABLE VIII.

0.1 C.C. SALIVA + 30 C.C. STARCH SOLUTION AT 36°.

Tube *A* contained old saliva papers.
The *B* contained fresh saliva papers.

	Control Saliva	Saliva from *A*	Saliva from *B*
3 hrs..........	Achromic in 60 min.	Achromic in 64 min.	Achromic in 66 min.
6 hrs..........	Achromic in 60 min.	Achromic in 74 min.	Achromic in 74 min.
24 hrs..........	Pink in 80 min.	Achromic in 66 min.	Achromic in 66 min.

The starch used in testing the twenty-four-hour control was twenty-four hours older than that used in testing the twenty-four-hour sets of *A* and *B*, which may account for the irregularity apparent in twenty-four-hour set.

This experiment was performed for the purpose of a comparison of papers presumably ptyalin-saturated (*A*), with fresh papers (*B*) as regards their action on 10 per cent. saliva. Both appear to have similar effect, a slightly greater avidity for ptyalin being suggested by the fresh papers at the end of three hours, after which they too appear to be saturated. A better comparison is afforded in Table XIII.

The paper bits in both tubes were now thoroughly washed until no trace of free ptyalin remained. The action of the papers was then tested as before.

TABLE IX.

Control	Iodine Reaction	Papers from	Iodine Reaction
Fresh paper control	Blue in 1 hr.		
Wash-water control (*A*).............	Blue in 1 hr. Red in 2 hrs. Achromic in 19 hrs.	*A* (old saliva papers)	Achromic in 1 hr.
Wash-water control (*B*).............	Blue in 2½ hrs.	*B* (fresh saliva papers)	Achromic in 1 hr.

In the meantime the papers in *B* were extracted by aeration with glycerin for thirty minutes. The test of the aerated glycerin for diastatic activity as compared with fresh glycerin resulted as follows:

15 c.c. of paper aerated glycerin + 15 c.c. 0.1 per cent. starch solution produced achromicity in 18 hours.

15 c.c. of fresh glycerin + 15 c.c. of 0.1 per cent. starch solution produced no inversion whatever in 18 hours.

EXPERIMENT VIII.

Added fresh glycerin to papers in *B* and fresh distilled water to *A*, and aerated for twenty-four hours. Tested wash-water of *A* and glycerin of *B* as before, controlling with fresh water and fresh glycerin.

TABLE X.

15 C.C. OF WATER AND OF GLYCERIN + 15 C.C. 0.1 PER CENT. STARCH SOLUTION AT 36°

Control	Color Produced by Iodine	Wash Liquid	Color Produced by Iodine
Water + starch....	Deep purple in 4½ hrs.	Water (*A*).......	Deep purple in 4½ hrs.
Glycerin + starch..	Blue in 1 hr.	Glycerin (*B*).....	Achromic in 1 hr.

Portions were tested every fifteen minutes in the above tests. Thus with twenty-four hours' washing, water removed none of the retained ptyalin whereas glycerin extracted it.

The tubes were then drained and rinsed with distilled water, after which fresh water was added to *A* and fresh glycerin to *B*. They were aerated then for forty-eight hours, and tested as before.

TABLE XI.

Control	Color Produced by Iodine	Wash-Liquid	Color Produced by Iodine
Water + starch....	Blue in 4½ hours	Water (*A*)........	Blue in 4½ hours
Glycerin + starch..	Blue in 4½ hours	Glycerin (*B*)......	Achromic in 4½ h.

Even this attempt at extraction (seventy-two hours' aeration) with water failed to remove the ptyalin from its close retention by the filter paper, although the paper yielded its ptyalin readily to the glycerin. That the paper still retained its ptyalin is further shown by the following:

The glycerin was thoroughly removed from *B* by repeated washings with distilled water. *A* was drained, and a third control tube was set up, containing fresh papers, approximately equal in quantity to those in *A* and *B*. Water was placed in tubes *A*, *B*, and *C*, and all were aerated for one and three-quarter hours. A portion of the papers was then removed from *A* and *B*, and the amylolytic action tested; tests were controlled by paper bits from *C*. Controlled further with wash-water from *A* and *B* by adding 15 c.c. of each to 15 c.c. of 0.3 per cent. starch solution and digesting at 36°.

TABLE XII.

Control	Color Produced by Iodine	Saliva Paper	Color Produced by Iodine
Paper control......	Blue in 3½ hrs. Purple in 18 hrs.		
Wash-water of *A*..	Blue in 3½ hrs. Purple in 18 hrs.	*A* (water-washed)	Blue in 3½ hrs. Purple in 6 hrs. Achr. in 18 hrs.
Wash-water of *B*..	Blue in 3½ hrs. Purple in 18 hrs.	*B* (glyc. extracted)	Purple in 3½ hrs. Achr. in 18 hrs.

The liquids were examined only at the times indicated in the table.

After enough liquid was removed from each tube for the preceding test, the tubes and contents were rinsed with 2 per cent. saliva (the latter being filtered, before dilution, through a single thickness of ordinary filter paper). To each tube there were then added 25 c c. of the 2 per cent. saliva, and the contents were aerated for twenty-four hours, with interruptions at the end of three and six hours as before. The tubes contained approximately equal quantities of paper and were aerated side by side at equal rates.

Tube *A* contained old saliva papers, water-washed.

Tube *B* contained old saliva papers, glycerin-extracted.

Tube *C* contained fresh papers, water-washed.

At the end of three, six, and twenty-four hours, 1 c.c. aerated 2 per cent. saliva from each tube was added to 30 c.c. starch solution and digested at 34° to 37°. The results are tabulated below:

TABLE XIII.

AERATION	COLOR PRODUCED BY IODINE			
	Control	Tube *A*	Tube *B*	Tube *C*
3 hours.....	Achr. in 50 min.	Achr. in 7 hrs.	Achr. in 6 hrs.	Blue in 7 hrs.
6 hours.....	Achr. in 50 min.	Pink in 8 hrs.	Achr. in 6½ hrs.	Blue in 1¼ hrs.
24 hours.....	Achr. in 50 min.	Blue in 3 hrs.	Purple in 3 hrs.	Blue in 3 hrs.

It is seen from the above that with sufficient dilution of saliva all of the ptyalin can be retained by the filter paper in the aeration tubes, just as it can be removed from like solutions by filtration. The paper bits in *A* and *B* already contained fixed ptyalin, and on that account could not remove all the ptyalin from the 2 per cent. saliva, although a glance at the table shows that a very considerable quantity was removed. The paper in *C* so effectually removed the ptyalin that at the end of three hours' aeration 1 c.c. of the liquid could produce no effect on 30 c.c. of 1 per cent. starch solution in seven hours, whereas a corresponding quantity of the control saliva completely inverted 30 c.c. of the starch solution in fifty minutes.

That the ptyalin was not destroyed is made evident by the following:

The paper bits in Tubes *A*, *B*, and *C* were thoroughly washed as before, and approximately equal quantities of each were immersed in test-tubes containing 30 c.c. of 1 per cent. starch solution. Control tests were made with fresh paper as before. Wash-water controls as before. The tests with the wash-waters were checked with distilled water. The digestion was carried on at 35°.

TABLE XIV.

Water Control	Color Produced by Iodine	Paper Bits	Color Produced by Iodine
Distilled water.....	Blue in 18 hrs.	Control.......	Purple in 18 hrs.
Wash-water *A*.....	Blue in 18 hrs.	Tube *A*.......	Pink in 3½ hrs. Achromic in 18 hrs.
Wash-water *B*......	Blue in 3½ hrs. Pink in 18 hrs.	Tube *B*.......	Achromic in 3½ hrs.
Wash-water *C*.....	Blue in 18 hrs.	Tube *C*.......	Blue in 1 hr. Pink in 3½ hrs. Achromic in 18 hrs.

The liquids were examined only at times indicated in the table.

EXPERIMENT IX.

Two sheets of S. & S. hardened filter paper, No. 575, diameter 24 cm., were cut up into small bits (about 1 cm. square), crumpled, and placed in an aeration tube, which we will designate as *A*. A similar quantity of papers was placed in a second tube *B*. In a third tube *C* were placed glass beads, the height of the column of which equaled that of the paper in *A* and in *B*. The saliva was filtered through a single thickness of ordinary filter paper and 4 c.c. of it was diluted to 200 c.c. with distilled water. After rinsing the tubes with this 2 per cent. saliva, 60 c.c. of the same were placed in *A* and *B* respectively, while *C* received 40 c.c.

Tubes *A* and C were connected with the pump and aerated at the same rate. Tube *B* remained unaerated for two hours, and was then aerated for five minutes, after which it remained unaerated for two hours, and then was again aerated for five minutes. It now remained unaerated for the next eighteen hours, after which it was aerated for fifteen minutes. The saliva in

A, *B*, and *C* was tested at three-hour intervals, and the tests were controlled with unaerated 2 per cent. saliva, which was kept at room temperature under a black cloth.

TABLE XV.

1 C.C. OF 2 PER CENT. SALIVA + 30 C.C. 1 PER CENT. STARCH SOLUTION AT 38°.

(For the six-hour test a new starch solution was prepared.)

SALIVA	COLOR PRODUCED BY IODINE		
	3 Hours	6 Hours	24 Hours
Control	Achr. in 26 min.	Achromic in 50 min.	Achromic in 38 min.
A	Achr. in 62 min.	Deep purple in 1 hr.; achr. in 16½ hrs.	Purple in 3 hrs.; achr. in 4 hrs.
B	Achr. in 62 min.	Blue in 1 hr.; achr. in 16½ hrs.	Blue in 4 hrs.; deep purple in 24 hrs.
C	Achr. in 26 min.	Achr. in 50 min.	Achr. in 32 min.

From the above table it will be seen that aeration over beads has no effect, and that fixation is more rapid when the liquid is not continuously agitated.

The papers in *A* and *B* were then washed by aerating for twenty-four hours with distilled water, which was frequently changed. The inverting action of the washed papers was tested as before.

TABLE XVI.

Paper Bits	Color Produced by Iodine
Control	Blue in 5¼ hrs.
Saliva paper *A*	Blue in 2 hrs.; light pink in 5¼ hrs.
Saliva paper *B*	Blue in 2 hrs.; achromic in 5¼ hrs.

DIALYSIS OF PTYALIN THROUGH COLLODION SAC.

Gorsline,[12] working in this laboratory, showed that such substances as starch and albumin can pass through the collodion membrane, and suggested the possibility of dialyzing enzymes. According to Rodet and Guéchoff,[13] the collodion wall does not act as a perfect filter. They say:

> To be acquainted with the value of collodion sacs in bacteriological experiments it is quite necessary to know whether the membrane of collodion constitutes a filtering septum, retaining only formed particles, and allowing to pass indiscriminately all soluble matters, or whether, endowed with properties intermediate between those of such a filtering wall and those of a semipermeable membrane, it allows soluble substances to pass more or less, and in a varying degree, according to their nature and the size of their molecules.

> We have wished first to see by an experiment *in vitro* if collodion is perfectly permeable to bodies of different molecular weights, such as sugar, peptone, albumin. . . . . Three similar sacs were filled with distilled water, and were plunged into test-tubes containing several cubic centimeters, one of a solution of glucose, another of a two per cent. aqueous peptone solution, and the third of blood serum. After twenty-four hours' sojourn at laboratory temperature special reactions were noticed in the contents of the sacs. The contents of the first reduced Fehling's solution abundantly; the sugar had traversed the wall of the collodion. The third sac gave the albumin reactions; but the content of albumin was very feeble, estimated practically 500 times less than that of the serum in which the sac was immersed; the albumin had traversed the wall but feebly. As to the contents of the second sac, the peptone reaction was not found there; it is, however, quite reasonable to suppose that it had passed, but in a quantity insufficient to be detected by the reagents. This experiment shows that collodion does not comport itself as a perfect filter.

It is quite reasonable to suppose that the sacs used by Messrs. Rodet and Guéchoff in this experiment were not of the same degree of thickness—that the sac used in the peptone dialysis was thicker than that used in the dialysis of albumin. If albumin readily traverses the collodion wall, it is quite evident that the relatively simpler peptone should do likewise. The inequality of the sacs is probably due to the fact that those used were of a commercial variety, as indicated below. To continue:

> Further, we have made two experiments on animals, one with diphtheria toxin, the other with an alkaloid which it may be assumed must readily traverse the membrane—strychnine.
>
> Experiment with diphtheria toxin: A guinea pig (600 g.) received in its peritoneal cavity a collodion sac (commercial) inclosing 2 c.c. of broth containing 5 g. of diphtheria toxin, fatal at 0.05 g. for a 250 g. guinea pig. It survived. Another (500 g.) received an intraperitoneal injection of 0.25 g. (a dose one-half less than that of the preceding) of the same toxin. It died in less than forty hours.
>
> A dose of diphtheria toxin, much greater than the fatal dose, is thus perfectly tolerated in a collodion sac.
>
> Experiment with strychnine: A guinea pig (540 g.) received July 7 in the peritoneal cavity a collodion sac (commercial) with 0.75 c.c. of an aqueous solution containing ¾ mg. of sulphate of strychnine. The following day it showed nothing in particular; no contractions. It died on the 14th, but its death was not due to strychnine. The operation wound had been opened, and allowed the intestine to protrude; the body was supple; on the evening of the 13th there had been no contraction. The contents of the sac, withdrawn and injected subcutaneously into another guinea pig, did not cause death.

> Consequently the toxin diffused out of the sac, but so gradually as to be tolerated.
>
> . . . . We are convinced that the collodion wall is far from being as permeable to dissolved substances as bacteriologists have admitted up to the present, and as a consequence the different interpretations of experiments made on this principle deserve to be held in doubt.

These experiments are of interest in that they call attention to the imperfect permeability of the collodion wall—a fact which our own experiments will further substantiate. We are inclined to believe, however, that with sufficiently thin sacs the dialysis in the above experiments would have been more marked.

We wish further to call attention to the experiments of Crendiroupoulis and Ruffer,[14] who find the toxin of pyocyaneus is partially dialyzable through the collodion wall, but not through walls of too great thickness. They state further that the comparison of the toxin filtered through the bougie with the toxin dialyzed through collodion shows a difference in pathogenic energy quite in favor of the former. They have, however, been able to produce with dialyzed toxin all the symptoms of pyocyaneus intoxication without exception, provided the quantity injected be sufficiently large.

The sacs employed in the following experiments were made according to the method described by Gorsline (*loc. cit.*). They were about 2½ inches in length and ½ inch in diameter, and were made by turning the tube used in making the sacs twice in dilute collodion, producing a sac of extreme thinness. These sacs were free from air bubbles, opaque areas, or flaws of any nature, were practically invisible when immersed in water, and collapsed immediately on emptying. Each sac was tested for possible leaks by immersing while empty in distilled water and distending with air under pressure. Those sacs showing evidences of leakage were discarded.

EXPERIMENT X.

Seven thin collodion sacs were prepared as above described. Each was mounted on a glass tube, some three inches in length, made by cutting off the end of a small test-tube. The sac was slipped over the end of this tube for a distance of a half-inch, and then contracted on the glass by rotation over a narrow flame, as described by Novy.[15] This joint was finally recoated with collodion. As stated above, each sac was tested for leaks before it was used.

A quantity of saliva was filtered through a single thickness of ordinary filter paper. In each of six sacs, labeled 1–6 inclusive, were placed 5 c.c. of this saliva. In the control sac, marked *C*, were placed 5 c.c. of same saliva, previously boiled for five minutes to destroy the enzyme. Each sac was immersed in 20 c.c. (Sac 5 in 25 c.c.) of a 0.01 per cent. starch solution, contained in a test-tube on foot. In no sac did the liquid reach the joint, thereby insuring impossibility of capillary escape of liquid from sac.

The tubes containing these sacs were set aside in an incubator at 37°. At the end of three hours 2 c.c. of the liquid were withdrawn from each tube and tested with several drops of dilute iodine with the following results:

TABLE XVII.

Contents of Tube	Reaction Produced by Iodine
C	Blue
1	Achromic
2	Achromic
3	Achromic
4	Achromic
5	Pink
6	Achromic

The contents of Tube 5 and of the control tube were again tested at the end of the fourth hour; the latter gave a blue, starch reaction, while the former was achromic.

The sacs were again tested and found to be free from leaks. The only way by which the inversion ef the starch could have been brought about was by the dialysis of the ptyalin through the collodion membrane.

Further similar experiments gave identically the same results and clearly demonstrated that ptyalin will dialyze through the collodion membrane.

The next point was to determine whether or not ptyalin could be filtered through such a membrane. For this purpose the following experiment was made:

EXPERIMENT XI.

A thin collodion sac was attached, in the manner above described, to a glass tube passing through a rubber stopper. The joint was further strengthened by winding with a silk thread, and again recoating with collodion. The sac was of the same thickness as those used in the above dialysis experiments. The empty sac was tested under water by forcing air into it at 3 inches' pressure, and was found to be free from leaks. The sac thus mounted was placed in a test-tube on foot, provided with a side arm. The rubber stopper and the mouth of the tube formed an air-tight joint. Five cubic centimeters of the undiluted saliva, previously filtered through a single thickness of ordinary filter paper, were then introduced into the sac. The side arm of tube was connected with a gently working aspirator, and a suction pressure of 2½ inches was maintained for one and one-half hours. During this time droplets of perspiration appeared on the outer side of the distended sac, slowly became confluent, collected in drops at the lower end of the sac, and fell to the bottom of the test-tube. In this way 1.5 c.c. of the contents of the sac were drawn through the wall and collected at the bottom of the test-tube. The filtering liquid and the control were protected during the process against the action of light by covering with black cloth.

One cubic centimeter of this filtrate was added to 30 c.c. of a 1 per cent. starch solution. An equal quantity of the control or paper-filtered saliva was added to a similar amount of starch solution. A third tube, containing 30 c.c. of the starch solution, served as a blank control. These tubes were set aside at 37–38°, and portions of the liquid were removed, from time to time, from each tube and tested with iodine. The result was as follows:

1 c.c. control + 30 c.c. starch solution — achromic in 20 min.
1 c.c. filtrate + " " " — blue in 22 hrs.
Starch control — blue in 22 hrs.

This experiment shows that the ptyalin was completely retained by the collodion filter.

The filter sac employed above was well washed, and then 5 c.c. of fresh filtered saliva were introduced. It was then immersed in 20 c.c. of 0.01 per cent. starch solution and placed in an incubator at 37°. The experiment was controlled with a sac similarly immersed containing 5 c.c. of boiled saliva. In a control test with iodine at the beginning of the dialysis the starch gave a deep blue color. At the end of three hours the starch in both tubes showed complete inversion. The fact that the control or heated saliva behaved thus showed that the boiling was insufficient to destroy its activity.

The sacs were again tested and found free from leaks. Thus, the same sac which refused passage to ptyalin, when used as a filter, allowed the enzyme to pass through it readily by dialysis. This fact is further substantiated by the following similar experiment:

Some saliva was placed in a new thin collodion sac and aspirated as before. Three and two-fifths c.c. of a water-clear liquid filtered through in one and one-half hours at a pressure varying from 2¾ to 3¼ inches. This filtrate was tested as before at 38°.

1 c.c. of control saliva + 30 c.c. of 1 per cent. starch solution—achromic in 30 min.
1 c.c. saliva filtrate + 30 c.c. 1 per cent. starch solution—blue in 45 hrs.
Blank control (30 c.c. of starch solution) blue in 45 hrs.

The control was made with the unfiltered saliva remaining in the sac. Therefore the activity of the ptyalin was not destroyed by the action of the collodion wall or by the pressure. The sac was tested before and after use at a pressure of 3¼ inches, and was found to be free from leaks. It was then employed for a dialysis test, for which purpose 5 c.c. of fresh saliva were introduced into the sac, which was then immersed in a test-tube on foot containing 20 c.c. of a 0.01 per cent. starch solution. A control sac similarly immersed, containing 5 c.c of saliva previously boiled for five minutes, was also set aside. The tubes were placed in the incubator at 37°. An initial control test with iodine gave a deep-blue color. At the end of one hour and at the end of one hour and forty minutes portions of starch were removed from each tube and tested with iodine with the following results:

TABLE XVIII.

	Control	1 Hour	1 Hr. 40 Min.
Used sac....................	Blue	Pink	Achromic
Control sac.................	Blue	Blue	Blue

The foregoing experiments demonstrate the ready dialysis of ptyalin through the collodion sac, and the perfect impermeability of the latter to ptyalin when used as a filter. This impermeability is not due to fixation, for if the collodion wall could fix ptyalin, dialysis could not occur.

The fact that ptyalin is retained from saliva on passage of the latter through hardened filter paper; that it is not retained by the Berkefeld bougie; that it is not affected by aeration; that paper bits which have been immersed in saliva, when washed entirely free from saliva, will yet invert starch; that this paper will not give up this property on washing with water, nor yield to the water any diastatic property, and that it yields its retained ptyalin readily to glycerin, goes to show that pytalin is *fixed* by hardened filter paper.

TAKA DIASTASE.

FILTRATION THROUGH PAPER.

The solutions of taka diastase (Parke, Davis & Co.), of a dilution of 1:4,000 were first filtered through a single thickness of ordinary filter paper. They were then subjected to filtration through eight double filters of hardened filter paper, and each filtrate was tested in the manner as given under ptyalin. In the several filtrations tried the "8 × 2" filtrate showed itself but little, if indeed at all, weaker in activity than the original control. Inasmuch as this 1:4,000 solution was but half as strong as a 10 per cent. solution of ordinary saliva, it was not deemed necessary to work with greater dilutions.

Experiments in the aeration tube likewise failed to demonstrate any fixation, although the activity of the enzyme was decreased by the aeration. This weakening was due either to the aeration itself, or to the surface action of the paper and the beads over which it was aerated, or to both.

The enzyme passed through the Berkefeld filter at vacuum pressure as readily as it passed through the "8 × 2" filters.

DIALYSIS.

The following, one of several similar experiments, indicates the dialysability of taka diastase:

Four thin collodion sacs were prepared and mounted as previously described, and labeled *C*, 1, 2, and 3. In each of 1, 2, and 3 were placed 5 c.c.

of a 1:1,000 distilled water solution of taka diastase, previously filtered through a single thickness of ordinary filter paper. In *C* were placed 5 c.c. of the same solution, previously boiled for five minutes. This served as control solution. Each sac was immersed in a test-tube on foot containing 20 c.c. of a 0.01 per cent. starch solution, and the whole was then placed in the incubator at 36° for the purpose of dialysis. A control test of the starch before immersion of the sacs gave a deep-blue coloration on addition of iodine. Portions of 2 c.c. were removed from each of the test-tubes at the end of three, six, nine, and twenty-four hours, and were tested with dilute iodine to observe progress of digestion, if any. The results obtained are given in the following table:

TABLE XIX.

Time	Tube *C*	Tube 1	Tube 2	Tube 3.
3 hrs.	Blue	Blue	Blue	Blue
6 hrs.	Blue	Light blue	Light blue	Light blue
9 hrs.	Blue	Pink	Pink	Pink
24 hrs.	Pink	Achromic	Achromic	Achromic

The sacs were tested before and after dialysis, and were found free from leaks.

Taka diastase therefore dialyzes, though slowly, through the collodion sac.

FILTRATION THROUGH THE COLLODION SAC.

The following table gives the results of several filtrations of taka diastase through thin collodion sacs, under conditions similar to those given in connection with ptyalin. The taka diastase was employed in a 1:1,000 solution in 0.7 per cent. NaCl. The solution was passed through a single thickness of ordinary filter paper before collodion filtration. Controls were made in each instance from the unfiltered liquid remaining in sac after filtration. The controls and filtrates were tested by adding 1 c.c. of the liquid to 30 c.c. of a 1 per cent. starch solution and digesting in water bath at 37°. A blank control was made in each instance with 30 c.c. of the starch solution. The sacs were tested at a pressure of three inches and were found to be free from leaks before filtration.

TABLE XX.

No.	FILT. PRESSURE	TIME OF FILT.	AM'T FILTERED	COLOR REACTION WITH IODINE OF		
				Starch Control	Diastase Control	FILTRATE
	(Ins.)	(Hrs.)	(c.c.)			
1.......	2½-3½	2	3.8	Blue in 19 hrs.	Achr. in 2-2½ hrs.	Purple in 6 hrs. Achr. in 19 hrs.
2.......	2-3	2	4.2	Blue in 17 hrs.	Achr. in *ca.* 3 hrs.	Purple in 3½ hrs. Achr. in 17 hrs.
3.......	2-3½	1½	3.5	Blue in 24 hrs.	Achr. in 1 hr. 40 min. -2 hrs. 50 min.	Achr. in 22-24 hrs.
4.......	3⅛	1½	5.0	Blue in 24 hrs.	Achr. in *ca.* 3 hrs.	Achr. in 18-24 hrs.

The sacs used in filtrations 2, 3, and 4 were tested after filtration and found to be free from leakage. The sac used in filtration 1 showed leakage at 2½ inches.

The sac used in filtration of No 2, after thorough washing, was filled with 5 c.c. of the 1 : 1,000 diastase solution and immersed in 20 c.c. of a 0.01 per cent. starch solution. In another sac were placed 5 c.c. of the same solution, previously boiled, and this was immersed similarly, and served as a control. Both sacs were placed in the incubator at 37°. A control or initial test of the starch solution gave a blue reaction with iodine. At the end of one and one-half hours 2 c.c. of the starch solution were removed from each tube and tested with iodine. The starch in the control tube gave a deep-blue color, whereas that in the tube containing sac No. 2 gave an achromic reaction.

The sac, after the dialysis, was tested at 3 inches' pressure and was found to be free from leakage.

It will be seen from the experiment that, although the sac greatly retarded the passage of taka diastase, by filtration, it permitted a ready dialysis of the enzyme.

It has been shown that ptyalin is totally retained by the collodion filter, but taka diastase passes through to a considerable extent. It seemed possible that the retention of ptyalin was due to the presence of mucin, and if such was the case, a similar behavior could be expected of taka diastase under like conditions. The following experiments were made in order to show the effect of mucin:

A quantity of saliva which had been filtered through a single thickness of ordinary filter paper was boiled for five minutes in order to destroy the ptyalin. After cooling, 10 c.c. of this saliva were added to 10 c.c. of a 1 per cent. taka diastase solution and thoroughly mixed. The mixture was filtered through a collodion sac in the manner previously described. The filtrate and the control were tested by adding 1 c.c. of the liquid to 20 c.c. of the 1 per cent. starch solution. Blank controls were made as before. The following table gives the results of two such filtrations:

TABLE XXI.

No.	Filt. Pressure	Time of Filt.	Am't. Filtered	Iodine Reaction of		
				Starch Control	Diastase Control	Filtrate
	(Ins.)	(Hrs.)	(c.c.)			
1.......	3-3¼	1½	3.3	Blue in 20½ h.	Achr. in 6 hrs. +	Purple in 6 hrs. Achr. in 20½ hrs.
2.......	3¼	1½	3.8	Blue in 20 hrs.	Purple in 3 hrs. Achr. in 17½ hrs.	Deep purple in 3 hrs. Red in 20 hrs.

At the close of the experiments the sacs were tested at a pressure of 3¾ and 5 inches respectively, and were found to be free from leakage.

The preceding filtrations were with mixtures of boiled saliva and taka diastase. The two following experiments were made with raw saliva and 1 per cent. taka diastase in equal parts. Inasmuch as ptyalin does not filter through the collodion sac, a positive result must be due to the passage of taka diastase, notwithstanding the presence of mucin and other saliva constituents.

EXPERIMENT I.

Three cubic centimeters of the mixture filtered through a sac in 1 hour at a pressure of 2½-3½ inches. The sac was tested after filtration and showed no leak at 5 + inches.

Two cubic centimeters of this filtrate added to 20 c.c. of a 1 per cent. starch solution and left at 36° gave a purple test in 5 hours and an achromic reaction in 21 hours.

The blank control (20 c.c. of a 1 per cent. starch solution left at 36°) gave a blue reaction at the end of 21 hours.

The activity of the mixture in the sac was tested by adding 2 c.c. to 20 c.c. of a 1 per cent. starch solution and left at 36°. An achromic reaction was obtained in 7 minutes.

EXPERIMENT II.

Three cubic centimeters of the mixture filtered through a sac in 1 hour at a pressure of 2¼-3¼ inches. No leak could be detected in the sac after filtration, even at 6 inches' pressure.

Two cubic centimeters of this filtrate were added to 20 c.c. of the starch solution and left at 36°. An achromic reaction was obtained in 32 to 42 minutes.

The blank control (20 c.c. starch solution at 36°) gave a deep purple at the end of 42 minutes.

Two cubic centimeters of the mixture remaining in the sac were added to 20 c.c. of the starch solution at 36°. Iodine gave an achromic reaction in 40 minutes.

These experiments show conclusively that the presence of mucin does not prevent the passage of taka diastase through the collodion filter. It is probably also without effect on the ptyalin molecule.

Summing up, it will be seen that taka diastase is not removed from its solution by filtration through paper; it passes readily through the Berkefield filter, partially through the collodion filter, and dialyzes through the collodion membrane. It is injuriously affected by aeration.

RENNET.

EXPERIMENT I.

Paper filtration.—That rennet is removed from its solution by filtration through hardened filter paper is evidenced by the following results:

The rennet solution used in this experiment was prepared by dissolving one tablet of the commercial preparation (Chr. Hansen) in 100 c.c. of physiological salt (0.7 per cent.) solution; the resulting solution was then filtered through a single thickness of ordinary filter paper. This clear solution was not used directly, but served for the preparation of a 1:40,000 dilution. In each experiment 200 c.c. of the 1:40,000 rennet solution, in physiological salt, were used. Of this amount 195 c.c. served for the purpose of filtration, and 5 c.c. for the control tests. The rennet activity of each filtrate was tested by adding 1 c.c. of each to 5 c.c. of fresh milk in a test-tube kept at about 37° in a water-bath and observing the time required for coagulation. The rennet experiments covered a number of days, and fresh dairy milk was obtained each morning for that day's work. The filtering liquids as well as the controls were kept under black cloth. A common control was used for the "6×2" and "8×2" filtrates. The filter paper employed was ordinary hardened filter paper cut into squares of 15 cm. each.

TABLE XXII.

1 c.c. OF FILTERED 1:40,000 RENNET SOLUTION + 5 c.c. MILK AT 36°.

No.	DURATION OF FILTRATION	DIGESTION TEMP.	TIME REQUIRED TO PRODUCE COAGULATION BY						
			"2×2"		"4×2"		"6×2"		"8×2"
			Control	Filtrate	Control	Filtrate	Control	Filtrate	Filtrate
	Min.		Min.	Min.	Min.	Min.	Min.		
1...	110	36°	58	85-135	45-55	238	52	5 hrs. 15 min.	Neg. 18 hrs.
2...	130	34-37°	26-32	52	27	94-123	25	Slight Coag. 16 hrs.	Slight Coag, 16 hrs.
3...	170	37°	90	152-197	94-109	110-155	73-103	5-6 hrs	5-16 hrs.

The experiments tabulated above clearly show that successive filtrations of a rennet solution rapidly decrease its activity. In order to determine whether or not the removal of the enzyme was due to fixation, aeration over crumpled pieces of filter paper was resorted to as in the case of the ptyalin experiments already described.

EXPERIMENT II.

Effect of aeration.— In these experiments the liquid was aerated in the special tube mentioned heretofore, over bits of filter paper, glass beads, and garnets. It was found that after three hours' aeration, either over crumpled paper bits or over glass beads, the liquid within the tube was without any coagulating action. The fact that the glass beads exerted the same action as the bits of paper indicated that the enzyme was destroyed and not merely fixed or retained by the paper. Moreover, all attempts at extraction with glycerin of enzyme possibly fixed by the paper bits produced negative results.

In order to make sure that the rapid destruction of the enzyme was not due to the alkalinity resulting from the action of the water on the walls of the tubes, on the beads themselves, or on the plug of glass-wool employed in keeping the paper bits in the tubes, the experiments were repeated with aeration tubes of Bohemian glass. One of these was filled with thoroughly cleansed garnets in place of the beads and the glass-wool. A second tube was filled with crumpled bits of paper, while as a further check, a third aeration tube was set up as a control in which the liquid was aerated alone, that is, not over garnets or paper.

Under these conditions the same ready destruction of the enzyme was obtained. In the control aeration tube the destruction was less rapid, six or more hours being required to destroy totally the enzyme, whereas over paper or garnets the rennet was destroyed in two to three hours. Control tests made with the unaerated rennet solution showed unimpaired activity during the time of these experiments. Moreover, it should be stated that tests were made with phenolphthalein of all liquids after aeration and showed an absence of alkalinity.

These facts then indicate that rennet in solution is readily destroyed by aeration, which destruction is hastened by the surface action of paper bits or garnets.

Effect of Berkefeld filters.—The retention of enzymes by porous porcelain filters has been observed in numerous instances. Gautier[16] called attention to the fact that, in the filtration of gastric juice through the porcelain bougie the pepsin was largely retained on the filter. Fermi and Pernossi[17] later experimented on the passage of trypsin and pepsin through the Chamberland bougie. They filtered a 1:500 solution of trypsin, containing five per cent. NaCl, through the bougie at a pressure of two atmospheres, and found a decrease of about one-half in the activity of the filtrate as compared with that of the original solution. The filtration of a 1:200 solution, containing no NaCl, caused a loss of about one-fourth of its strength, while the filtration of a 1:200 solution containing two per cent. of Na_2CO_3 resulted in the loss of about half of the strength of the solution.

In order to show that the decrease in activity was not due to the removal of undissolved trypsin which might be present, they filtered a 1:200 solution of the enzyme through the bougie at one atmosphere pressure. This filtrate was again filtered through the bougie, and the resulting filtrate was passed through the filter once more, giving thus a triple filtration of the solution. They tested their controls and the filtrates by addition to gelatin, and found that the successive filtrates progressively decreased in activity. This fact was taken to prove that the weakening of the filtrate was not due to the retention of undissolved particles.

Setting aside the possibility that the trypsin could be destroyed by the filtration process, they reached the conclusion that the loss of the activity of the filtered solution was due to the retention of the molecules by the filter. To quote their words:

> "We come to the conclusion that trypsin passes through the porcelain filter in such a way that, when one repeats (four to five times) the filtration of the same solution, the trypsin remains entirely on the filter."

They also observed a weakening in the activity of solutions of pepsin by refiltering through the porcelain bougie.

Achalme[18] also noted the retention of pancreatin by the bougie, but according to his statement the pancreatin loses only about 25–35 per cent. of its diastatic power, when filtered at a temperature of 35–38°.

Lea (*loc. cit.*) observed the retention of the urea ferment by the porous battery cell. Miquel (*loc. cit.*) was for a long time unable to pass urase through the porcelain bougie, but finally succeeded in obtaining a passage of the ferment after displacing the atmospheric air in the pores of the filter with illuminating gas.

Fermi[19] observed the total retention by the porcelain filter of the inverting enzyme of B. megaterium.

Buchner[20] succeeded in obtaining a partial passage of zymase through both the Berkefeld and Chamberland bougies. Fernbach[21] has shown that the sucrase of Aspergillus niger is totally retained by the porcelain bougie, whereas the sucrases of the Champagne yeast, of Saccharomyces Pastorianus, and of pale ale pass *en totalité* through the filter. According to Effront,[22] the invertin of yeast passes the Chamberland filter, whereas that of aspergillus does not. Ehrlich and Morgenroth[23] have pointed out that the complement in goat's blood causing the solution of rabbit corpuscles is retained on the Pukallfilter.

Vedder,[24] in working on the bacteriolytic complements in the rabbit's blood, filtered the serum through Chamberland and Berkefeld filters, with the following results:

> The complements for all the organisms tested (B. typhosus, B. coli communis, B. dysenteriæ, and S. pyogenes aureus) may be separated from the serum by filtration through a Chamberland filter; but by passage through a Berkefeld filter the complements for certain organisms are retained (B. coli communis, S. pyog. aureus, and B. dysenteriæ, partially), while those for others (B. typhosus, B. dysenteriæ partially) pass through — a further indication of the multiplicity of the bacteriolytic complement. By filtration through a Chamberland filter it is possible to separate the agglutinins from the bactericidal substances of a serum.

We have known but little hitherto about the filtration of rennet. According to Oppenheimer,[25] Lea[26] found that vegetable as well as animal rennet was retained by the kaolin filter. In the reference cited by Oppenheimer we find no authority for such a statement. Lea found a milk-coagulating ferment in the plant Withania coagulans. In order to decolorize its solution he added finely divided kaolin, which addition resulted in the weakening of the enzyme. No mention is made of filtration through kaolin. In view of a more recent paper by Lea (and Dickinson),[27] in reply to Fick's theory as to the action of rennet, it seems improbable that he has witnessed any such retention. We quote the following experiments:

> Some milk was placed in a narrow (2 cm. wide) porous battery cell made of extremely thin earthenware. The cell was then immersed in a beaker of rennin solution, so that the level was the same inside and outside the cell, and digested at 40° for seventeen hours. At the end of this time there was not a trace of clot in the milk or on the inner surface of the cell. The experiment was repeated for a period of twenty-seven hours, with the same result. It appeared to us that if Fick's view is correct, then a clot might have been expected to form in the above experiment, if not throughout the whole mass of the milk, at least along the inner walls of the cell; but no trace of a clot was observed. It would perhaps, however, be unwise to lay too much stress on this experiment, for we know but little of what is happening in the pores of the cell's wall when it is used as a septum between two different fluids. But it is scarcely conceivable that the rennin should not have come in contact with the milk, bearing in mind that such a cell is permeated by pores through which a filtration of fluids can be carried on under pressure.

Lea and Dickinson did not attempt, however, to filter rennet solution through these pores under pressure.

Roberts[28] (1880) found that the brine extract of pancreas when filtered under vacuum pressure through porous earthenware possessed an undiminished faculty for curdling milk, but it had almost no power of dissolving the curds. The curdling ferment had evidently traversed the earthenware freely, but only traces of trypsin had passed through.

Conn[29] (1892) separated rennet from the proteolytic enzyme of certain bacilli (B. mesentericus vulgatus?) by mixing the products of the organisms with milk and allowing coagulation to occur. This coagulum was broken up, then well shaken with distilled water, and the resulting solution filtered through porcelain. H_2SO_4 was added until the liquid contained 0.1 per cent., and NaCl was then added to saturation. A white granular-looking scum floated to the top, which was found to be almost pure rennet. The proteolytic enzyme remained behind in the brine.

Korschun[30] recently published a paper from which it appears that he has filtered rennet solution through both Berkefeld and Chamberland bougies. He gives no description of his process except that he used a little Berkefeld bougie. He finds a partial, but not complete, retention of the coagulating enzyme by the filter.

We know but little of the filter employed by Roberts. If, however, his results are correct, they give us an important distinction between pancreatic and gastric rennet; the passage of the rennet employed by Conn may be due to the fact that it is of vegetable origin. As regards Korschun's work, we are inclined to believe that he used imperfect bougies, or a rennet differing in properties from that employed by us. Our own experiments will show that the Berkefeld bougie, in perfect condition, will deny passage to the enzyme rennet, whether the filtration force be simply gravity, or 100 pounds' pressure to the square inch. On the other hand, old used bougies become more or less permeable.

EXPERIMENT III.

In this, as in all succeeding experiments, the rennet solution was filtered, either in the dilute form in which it was used, or previous to dilution, through one thickness of ordinary thin filter paper to insure absence of undissolved particles of rennet. The rennet was either that of Chr. Hansen or of Parke, Davis & Co., which commercial preparations were found to be identical in strength and behavior. Seven-tenths per cent. NaCl was used as the solvent for these solutions. The bougies were sterilized in every instance before each individual filtration. It was observed that new Berkefeld bougies contain a considerable quantity of alkali, which passes into solution in the liquids traversing the bougie. Hence, before each individual filtration the bougie was washed by drawing through it hot distilled water, until the wash-water failed to give an alkaline reaction to phenolphthalein. The bougie was then cooled down to room temperature by passage of cold distilled water. Every enzyme filtrate was tested and found to be alkali-free. In the high-pressure filtration experiments the bougies were further washed by forcing distilled water through them at the pressure employed in the subsequent rennet filtration. The filtering apparatus employed was that described by Novy.[31]

Filtration by gravity.—One hundred and eighty cubic centimeters of a 1:10,000 rennet solution filtered through a Berkefeld bougie at gravity pressure in two hours. A portion of the solution was previously set aside to serve as control. The filtrate and control were tested by adding 1 c.c. of each to portions of 5 c.c. of milk. Blank controls of milk were made to indicate coagulating action, if any, of micro-organisms. The digestion temperature was 36°.

The tests which were made in this experiment resulted as follows:

1 c.c. of control enzyme + 5 c.c. milk gave coagulation in 23 min.
1 c.c. filtrate + 5 c.c. milk gave no coagulation in 6 hrs.
Milk control gave no coagulation in 6 hrs.

It appears, therefore, that Rennet does not pass through the Berkefeld bougie in gravity filtration.

EXPERIMENT IV.

Filtration at vacuum pressure. — The following tabulated set of filtrations was carried on by aspiration. In the first three filtrations the liquid was allowed to pass through the bougie immediately upon beginning of suction, the pressure increasing during the filtration process to the point indicated in the table. In the latter three filtrations the receiving flask was exhausted before admission of the liquid, so that the entire filtration was carried on at the pressure indicated in the table. Milk controls indicated in the table are blank controls consisting of merely 5 c.c. of milk, without addition of enzyme. The tests of controls and filtrates were made by adding 1 c.c. of the liquid tested to 5 c.c. of milk.

TABLE XXIII.

No.	Dilution	Pressure	Duration of Filtration		Amt. Filt.	Temp. of Digestion	Coagulating Action of		
							Control		Filtrate
							Milk	Rennet	
		Ins.	Min.	Sec.	c.c.				
1.....	1:20,000	26⅞	1	50	195	37.5–36°	....	Coag., 40–44 m.	Neg. in 20 hrs.
2.....	1:10,000	27½	1	30	195	40°5	....	Coag. in 20 m.	Neg. in 8 hrs.
3.....	1:10,000	27½	0	62	196	39°	....	Coag. in 21 m.	Neg. in 2 hrs.
4.....	1:10,000	27⅛	1	30	195	36°	Neg., 2 h. 5 m.	Coag. in 19 m.	Neg., 2 h. 5 m.
5.....	1:10,000	26⅛	0	75	195	35°	Neg. in 11 hrs.	Coag. in 21 m.	Neg. in 11 hrs.
6.....	1:10,000	27⅞	0	85	195	35°	Neg. in 7 hrs.	Coag. in 23 m.	Neg. in 7 hrs.

As above mentioned, Achalme found that pancreatin loses only 25–35 per cent. of its activity when filtered through the porcelain bougie, provided the solution is heated to 35–38°. In the following filtrations not only were the solutions warmed to the temperature indicated in the table, but the filters were also heated to the same temperature, respectively, by the repeated passage of warmed distilled water through the bougie. Thus, in the following filtrations the solutions of 37, 43, and 46° passed through filters of the same respective temperatures. To determine the effect, if any, on the enzyme of warming, controls were made with the liquid both at room temperature and at filtration temperature.

TABLE XXIV.

No.	Dilution	Pressure	Time of Filtration	Amt. Filt.	Temp. of Filtration	Temp. of Digestion	Coagulating Action of		
							Control		Filtrate
							Room Temp.	Filt. Temp.	
		in.	sec.	c.c.			Coag. in	Coag. in	Negative in
1....	1 : 10,000	28	65	190	37°	37–35°	21 min.	21 min.	3 hrs. 50 min.
2....	1 : 10,000	27¾	....	190	43	39–37	20 min.	20 min.	16 hrs.
3....	1 : 20,000	26¾	110	190	46	37	46 min.	46 min.	4 hrs. 30 min.

An attempt made to filter at 52° with its corresponding increase in molecular motility, however, resulted in the destruction of the enzyme. In the four filtrations the liquids were gently warmed over the Bunsen flame, and then filtered immediately upon arriving at the desired temperature.

We have already called attention to the fact that Miquel succeeded in filtering urase through the porcelain bougie in an atmosphere of illuminating gas, whereas the enzyme failed to traverse the bougie in the presence of air. The following experiment is designed to demonstrate the behavior of rennet as regards filtration in an atmosphere of inert gas:

a) One hundred and ninety-five cubic centimeters of 1:10,000 rennet solution were filtered through the Berkefeld bougie at 28 inches' pressure in 45 seconds in an atmosphere of air. Controls and filtrates were tested as before.

1 c.c. control + 5 c.c. milk at 36°—coagulated in 20 min.
1 c.c. filtrate + 5 c.c. milk at 36°—coagulated in about 5 hrs.
Milk control negative in 5 hrs.

b) Scrubbed and sterilized the bougie used in *a*. Into the mouth of the filter globe inserted a rubber stopper with two apertures, one of which carried a tube connected with the illuminating-gas supply, the other carrying the tube of a separatory funnel. Closed the cock of the funnel and poured into the latter 190 c.c. of 1 : 10,000 rennet solution. Exhaustion was produced in the globe, bougie, and receiver, and gas was then admitted into the apparatus until the pressure fell to about five inches. Exhaustion was again produced, and gas was readmitted until the pressure fell to about five inches. The rubber connection between the receiver and the cylinder was then clamped, and the receiver exhausted, gas in the meantime continuously passing into the globe and bougie. When the receiver was exhausted, the clamp was released, and at the same time the enzyme solution was allowed to pass from the separatory funnel into the globe, leaving behind enough liquid in the funnel, however, to prevent entrance of air into the globe. This remaining liquid was also used for the control test. Liquid thus passed through the bougie in an atmosphere of illuminating gas, and in the absence of air.

Pressure, 27⅞ inches; time of filtration, 60 seconds; amount filtered, 185 c.c.

1 c.c. control + 5 c.c. milk at 36 degrees—coagulated in 23 min.
1 c.c. filtrate + 5 c.c. milk at 36 degrees—coagulated in 6–7 hrs.
Milk control—coagulated in 6–7 hrs.

c) Scrubbed and sterilized the bougie and washed the same thoroughly. Then filtered 195 c.c. of 1 : 10,000 rennet solution in an atmosphere of air at a pressure of 28⅛ inches in sixty seconds.

1 c.c. control + 5 c.c. milk 36°—coagulated in 15 min.
1 c.c. filtrate + 5 c.c. milk 36°—coagulated in 6–7 hrs.
Milk control—coagulated in 6–7 hrs.

It does not seem, after comparison with filtrations *b* and *c*, that any of the enzyme passed through the bougie in filtration *a*. The milk used in this experiment was undoubtedly very rich in organisms, and had we kept the milk control under observation a few minutes longer, we should no doubt have observed coagulation. As before mentioned, milk was obtained fresh each morning for the work of the ensuing day, but it seems probable that on this particular day an aged milk was substituted.

Rennet, therefore, does not pass through the Berkefeld bougie in an atmosphere of illuminating gas. Is gas absolutely inert toward rennet, or is the absence of activity in the filtrate due to the destruction of the enzyme by the gas? The following experiment was made in order to answer this question:

In each of three test-tubes of Bohemian glass, each provided with a side arm, were placed 20 c.c. of a 1 : 10,000 rennet solution. The tubes were designated *A*, *B*, and *C*, respectively.

Through rennet in *A* gas was passed for five minutes; the rennet in *B* was aerated for five minutes, entrance of foreign particles into the tube being guarded against by a cotton plug in the side arm; the contents of *C* served as control. Each of these was tested, with the following results:

1 c.c. of control + 5 c.c. milk at 36°—coagulated in 23 min.
1 c.c. of *A* + 5 c.c. milk at 36°—coagulated in 21 min.
1 c.c. of *B* + 5 c.c. milk at 36°—coagulated in 21 min.
Milk control was negative.

Illuminating gas can therefore be said to be without effect on rennet solutions.

The foregoing experiments show that rennet solutions do not pass through the Berkefeld bougie at vacuum pressure, either at room temperature or at higher temperature, or in an atmosphere of illuminating gas.

EXPERIMENT V.

Filtration at fifty pounds' pressure.—The procedure of pressure filtration employed in the following experiment is that described by Novy (*loc. cit.*). Save in the first two filtrations, all filtered liquids were forced directly into the spherical receiver described in the text, thereby obviating the effect on the filtrate of possible leakage in the apparatus.

A. One-hundred and ninety-five cubic centimeters of a 1:10,000 rennet solution were subjected to filtration at 50 pounds' pressure through the Berkefeld bougie. The time of passage was about fifteen seconds. The tests of control and filtrate resulted as follows:

1 c.c. control + 5 c.c. milk at 34-35°—coagulated in 18 min.
1 c.c. filtrate + 5 c.c. milk at 34-35°—coagulated in 9-11½ hrs.

No milk control was made, but it is evident that no enzyme passed through the bougie.

The bougie was employed in the meantime in a filtration test at 100 pounds' pressure (Experiment VI*a*), after which it was used for another filtration at 50 pounds. One hundred and ninety-five cubic centimeters of the solution were filtered in about ten seconds.

1 c.c. control + 5 c.c. milk at 36°—coagulated in 23 min.
1 c.c. filtrate + 5 c.c. milk at 36°—coagulated in 40 min.
No milk control.

In the first filtration the bougie had previously been used for nothing but vacuum-pressure filtration, and no enzyme passed through the bougie at 50 pounds' pressure. An ensuing filtration at still higher pressure had evidently worn channels in the filter, and a second filtration at 50 pounds caused a retention of less than half of the enzyme.

B. In this test a new bougie was employed. One hundred and ninety cubic centimeters of a 1:10,000 rennet solution were filtered in eight seconds at 50 pounds' pressure.

1 c.c. control + 5 c.c. milk at 36°—coagulated in 24 min.
1 c.c. filtrate + 5 c.c. milk at 36°—negative in 2 hrs. 15 min.
Milk control—negative in 2 hrs. 15 min.

Observations not taken after two and a quarter hours.

In the meantime the bougie was used for four filtrations at 100 pounds' pressure. Then another filtration was made at 50 pounds. One hundred and ninety five c.c. were filtered in ten seconds. The tests resulted as follows:

1 c.c. control + 5 c.c. milk at 35°—coagulated in 21 min.
1 c.c. filtrate + 5 c.c. milk at 35°—coagulated in 32 min.
The milk control was negative.

One hundred and ninety-five cubic centimeters of a 1 : 10,000 rennet solution, made up from the same 1 : 1,000 solution as that used in the preceding filtration, were filtered through another bougie (not new) at 50 pounds' pressure in ten seconds:

1 c.c. control + 5 c.c. milk at 34.5°—coagulated in 22 min.
1 c.c. filtrate + 5 c.c. milk at 34.5°—coagulated in 2 hrs. 20 min.
The milk control was negative.

The preceding experiments go to show that repeated filtrations under high pressure through the same bougie cause a decrease in the retaining properties of the bougie. The following series of filtrations was designed to bring out this point more fully.

C. Six filtrations of a 1 : 10,000 rennet solution were made through the same bougie at 50 pounds' pressure. The bougie was sterilized before each filtration by heating in an autoclave in about two liters of distilled water to 136-140° C. Twelve hundred and twenty cubic centimeters of a 1 : 10,000 rennet solution were made up and filtered in the six portions indicated below. The tests of filtrates and control were made as in the preceding experiments Milk controls, without the addition of rennet, were also made as before. The digestion temperature was 35°.

TABLE XXV.

No.	Amount of Filtrates	Time of Filtration	Coagulating Action of		
			Control		Filtrate
			Milk	Rennet	
1.....	195 c.c.	9 sec.	Neg.	Coag. in 20 min.	Coag. in 1 hr. 45 min.
2.....	195 c.c.	10 sec.	Neg.	Coag. in 21 min.	Coag. in 2 hrs. 54 min.
3.....	190 c.c.	15 sec.	Neg.	Coag. in 21 min.	Coag. in 1 hr. 55 min.
4.....	190 c.c.	14 sec.	Neg.	Coag. in 21 min.	Coag. in 3 hrs. 5 min.
5.....	150 c.c.	12 sec.	Neg.	Coag. in 21 min.	Coag. in 5 h. 53m.-6h. 8m.
6.....	130 c.c.	8 sec.	Neg.	Coag. in 22 min.	Neg. in 7 hrs. 15 min.

These results were rather surprising in that they seemed to indicate a clogging up of the pores of the filter by the rennet molecules and other substances, instead of the opening up of fresh channels. It will be observed, however, that in the former experiments which led to this series the intermediate filtrations were at 100 pounds' pressure, and not at 50 pounds.

To determine the effect of the concentration of the solution upon the retention of the enzyme by the pores of the filter, the following experiment was performed. It tends to indicate that concentration is without effect in retarding the passage. If anything, it shows that the more concentrated the solution the more readily will the enzyme pass through.

D. Made up 250 c.c. of a 1:1,000 rennet solution and diluted 10 c.c. of this to 200 c.c. with 0.7 per cent. NaCl. Filtered 195 c.c. of the resulting 1:20,000 solution through the bougie used in *C*, at 50 pounds' pressure. Sterilized the bougie and 195 c.c. of the 1:1,000 solution were filtered through it at 50 pounds' pressure. Tests of filtrates and controls were made as before. The digestion temperature was 35°.

TABLE XXVI.

DILUTION	TIME OF FILTRATION	COAGULATING ACTION OF		
		Control		Filtrate
		Milk	Rennet	
1:20,000.....	11 sec.	Negative	Coag. in 34 min.	Coag. in 5¼–7⅛ hrs.
1:1,000......	11 sec.	Negative	Coag. in 2 min.	Coag. in 13 min.

Thus the passage through the bougie of rennet solutions at 50 pounds' pressure depends entirely upon the bougie employed. The enzyme is totally retained by a new bougie. When old bougies are used, the enzyme passes through in varying amounts, depending upon the previous use to which the filter has been put, and also upon the concentration of the enzyme solution.

EXPERIMENT VI.

Filtration at 100 pounds' pressure.—*a*) One hundred and ninety-five cubic centimeters of a 1:10,000 rennet solution were filtered through the Berkefeld bougie used in Experiment *A* of the 50-pound pressure series. The liquid passed through in seven seconds.

1 c.c. of control + 5 c.c. milk at 39°—coagulated in 16 min.
1 c.c. of filtrate + 5 c.c. milk at 39°—coagulated in 1½–1⅔ hrs.
No milk control was made.

This showed that there was a partial retention of the enzyme by the filter. The bougie was washed by drawing through it about two liters of distilled water. It was then immersed in 50 c.c. of milk in a large test-tube, which was placed in an incubator at 37°. Beside it was placed another test-tube containing 50 c.c. of milk to serve as a control.

The milk in the tube containing the bougie curdled at the end of six and one-half hours, whereas the control milk was still perfectly liquid. During the succeeding nine hours coagulation occurred also in the control tube, but at the end of that time the milk in the bougie tube showed a much more marked coagulation. The curd was solid and a clear transparent whey was present, whereas that of the control was creamy.

This experiment goes to show that the lessened activity of the filtrate is not due to the destruction of the enzyme, but that this is probably retained in an active condition in the pores of the filter.

b) In this test a new bougie was employed. The filtration of 195 c.c. at 100 pounds' pressure was not complete because the stopper blew out of the globe, but not, however, before 70 c.c. of the liquid had passed through. This passage was practically instantaneous.

1 c.c. control + 5 c.c. milk at 36° — coagulated in 17 min.
1 c.c. filtrate + 5 c.c. milk at 36° — negative in 7½ hrs.
No milk control was made.

This test shows a complete retention of the enzyme by the filter.

c) Through this same bougie the following series of filtrations at 100 pounds' pressure was carried on. After each filtration the bougie was washed and then immersed in 50 c.c. of milk. Duclaux[32] states that the Chamberland bougie immersed in milk will cause coagulation by surface action, layers of coagulum due to the rupture of the equilibrium of the milk particles by surface action being deposited on the bougie. In the following experiments, as in *a*, the action of the bougie was controlled by a sterilized Berkefeld bougie, similarly immersed.

TABLE XXVII.

(Digestion temperature, 36°.)

No.	Amt. of Filtrate	Time of Filtration	Coagulating Action of			Coagulating Action of Bougie	
			Control		Filtrate		
			Milk	Rennet		Control	Filter
1......	190 c.c.	5 sec.	Negative	Coagulated in 14 min.	Coagulated in 5 hrs.	Negative	Coagulated in 5 hrs. 57 min.
2......	194 c.c.	5-10 sec.	Negative	Coagulated in 21 min.	Coagulated in 1½ hrs.	Negative	Coagulated in 8 hrs. 30 min.
3	195 c.c.	5-10 sec.	Negative	Coagulated in 26 min.	Coagulated in 43 min.	Negative	Coagulated in 8 hrs. 30 min.

It is thus seen that rennet is retained by a new Berkefeld filter even when filtered under a pressure of 100 pounds, but successive filtrations through the same bougie render it more permeable.

EXPERIMENT VII.

A new Berkefeld bougie was rendered alkali-free, as before indicated. It was then used for a series of nine filtrations of a 1 : 10,000 rennet solution. These were made in three sets; the first set consisted of three filtrations at vacuum pressure, the second of a like number at 50 pounds' pressure, and the third at 100 pounds' pressure. For the first two sets two liters of a 1 : 10,000 rennet solution were made up. For the third set, performed on the next day, a liter of a fresh 1 : 10,000 solution was prepared. In each instance 195 c.c. were filtered, and 5 c.c. were set aside for control. The controls were subjected to the same light and temperature conditions as the filtering liquid. The control and the corresponding filtrate were tested at the same time. The tests were made as before at 35°, by adding 1 c.c. of the liquid to 5 c.c. of milk. Blank controls of the milk were made as before. *In no instance did the enzyme traverse the filter.*

TABLE XXVIII.

No.	Pressure	Time of Filtration	Coagulating Action of		
			Control		Filtrate
			Milk	Rennet	
1	27 in.	75 sec.	Negative	Coagulated in 18 min.	Negative in 13 hrs.
2	27 in.	75 sec,	Negative	Coagulated in 18 min.	Negative in 12 hrs.
3	27 in.	90 sec.	Negative	Coagulated in 18 min.	Negative in 9¼ hrs.
1	50 lbs.	16 sec.	Negative	Coagulated in 18 min.	Negative in 7¾ hrs.
2	50 lbs.	16 sec.	Negative	Coagulated in 17 min.	Negative in 6¼ hrs.
3	50 lbs.	17 sec.	Negative	Coagulated in 17 min.	Negative in 5¼ hrs.
1	100 lbs.	10–15 sec.	Negative	Coagulated in 17 min.	Negative in 11 hrs.
2	100 lbs.	*ca.* 10 sec.	Negative	Coagulated in 17 min.	Negative in 11 hrs.
3	100 lbs.	*ca.* 10 sec.	Negative	Coagulated in 17 min.	Negative in 9½ hrs.

When the 50-pound set was examined ten hours after the last observation indicated in the table, coagulation had occurred in both filtrate and in blank milk control tubes. The coagulum in the filtrate tubes did not resemble the rennet control coagulum, which formed, on standing a few hours, a thick curd, but resembled rather the creamy coagulum of the milk control.

At the end of twelve hours coagulation occurred simultaneously in both milk control and the filtrate tube of Filtrates 1 and 2 of the 100-pound set.

In every instance there is a total retention of rennet by the Berkefeld bougie at vacuum pressure, and, save in the case of old bougies, there is the same complete absence of activity in the filtrates of the 50-pound and 100-pound pressure filtrations.

The enzyme is not destroyed or altered by the bougie, but is merely retained mechanically by the pores of the filter. It is almost inconceivable that the enzyme, in an experiment such as *b* of the 100-pound filtrations, should become destroyed or chemically altered during the almost instantaneous passage of the liquid through the bougie.

That the enzyme is not destroyed is evident from the fact that the bougies used in the 100-pound pressure filtrations, when immersed in milk, readily cause coagulation. That the pressure itself is without effect on the enzyme is clear from the demonstration that the same retention is observed in gravity filtration as in that at 100-pound pressure.

This fact is further shown by the following: 10 c.c. of a 1 : 10,000 solution of rennet were placed in a Pfungst autoclave and subjected to a pressure of 100 pounds for one hour at room temperature. In the meantime, as a control, 10 c.c. of the same solution were kept in a black box. At the end of the hour the coagulating action of each liquid was tested at 36°, with the following results:

1 c.c. autoclave rennet + 5 c.c. milk — coagulated in 34 min.
1 c.c. control rennet + 5 c.c. milk — coagulated in 34 min.
A milk control was negative.

It follows therefore that pressure is without effect.

EXPERIMENT VIII.

The following experiment was made in order to determine the effect, if any, exerted by the material composing the bougie upon the dissolved rennet:

A Berkefeld bougie was ground up in mortar to a very fine powder, which was then sterilized. To the powdered bougie in a beaker 190 c.c. of a 1:10,000 rennet solution were added, and the mixture was agitated by means of a mechanical stirrer for five minutes. A portion of 10 c.c. of the 1 : 10,000 solution was set aside as a control under the same temperature and light conditions as obtained for the mixture.

After the stirring ceased, the suspended particles settled very slowly and evenly. At the end of five and one-half hours the mixture was still very turbid. Ten cubic centimeters of mixture were then centrifugated, as also was the control, for thirty minutes. With the still cloudy liquid the following tests were made at 36°:

1 c.c. mixture + 5 c.c. milk—coagulated in 61 min.
1 c.c. control + 5 c.c. milk—coagulated in 36 min.
The milk control was negative.

After again sterilizing the powder, this experiment was repeated. The mixture was stirred this time for ten minutes and allowed to settle for two and three-quarter hours, after which it was centrifugated for one hour. A control solution was kept under the same conditions. The mixture remained turbid as in the preceding trial. The tests were made at 36°:

1 c.c. mixture + 5 c.c. milk—coagulated in 57 min.
1 c.c. control + 5 c.c. milk—coagulated in 30 min.
The milk control was negative.

The decrease noted above in the activity of the rennet solution is no doubt due to the carrying down of the enzyme molecules by the sedimenting of the diatomaceous earth particles which make up the Berkefeld bougie.

EXPERIMENT IX.

It may be assumed that the behavior of the filter is due to the presence of some substance in the bougie which, when dissolved out by the rennet solution, inhibits the action of the enzyme.

If there is anything of an inhibiting nature in the undisintegrated bougie, this substance must clearly appear in the filtrate. The following tests show the filtrate to be perfectly free from any substance of an inhibitory nature:

One hundred and ninety-five cubic centimeters of a 1:10,000 rennet solution were filtered through a Berkefeld bougie at a pressure of 27⅞ inches in eighty-five seconds. Fifty cubic centimeters of filtrate were then added to 50 c.c. of unfiltered solution, and the mixture was subjected to mechanical stirring for ten minutes. Another portion of 50 c.c. of the unfiltered solution was set aside for control. The tests were then made at 35°:

1 c.c. mixture + 5 c.c. milk—coagulated in 46 min.
½ c.c. control + ½ c.c. distilled water + 5 c.c. milk—coagulated in 46 min.
1 c.c. control + 5 c.c. milk—coagulated in 23 min.
1 c.c. filtrate + 5 c.c. milk—negative in 7 hours.
The milk control was negative in 7 hours.

Repeated experiments produced the same results and confirmed the belief that the filtered liquid had no inhibitory action other than that of a mere diluent.

This fact was also shown in another way. One hundred cubic centimeters of the filtrate obtained in the above experiment were evaporated in a Roux vacuum desiccator at 37° to 10 c.c. The following mixtures were then made with a 1:10,000 rennet solution:

A = 5 c.c. evaporated filtrate + 5 c.c. rennet sol.
B = 5 c.c. distilled water + 5 c.c. rennet sol.
C = 1 c.c. evaporated filtrate + 5 c.c. rennet sol.
D = 1 c.c. distilled water + 5 c.c. rennet sol.

One cubic centimeter of each solution was then added to portions of 5 c.c. of milk, kept at 35°, with the following results:

1 c.c. *A* + 5 c.c. milk—coagulated in 58 min.
1 c.c. *B* + 5 c.c. milk—coagulated in 44 min.
1 c.c. *C* + 5 c.c. milk—coagulated in 34 min.
1 c.c. *D* + 5 c.c. milk—coagulated in 30 min.
The milk control was negative.

The controls *B* and *D* are not strictly comparable to the test mixtures since they were made with distilled water, whereas the evaporated filtrate, owing to the fact that the rennet solutions were made up in physiological salt, contained seven per cent. of NaCl. To show the effect of seven per cent. of NaCl on a 1 : 10,000 rennet solution, the following mixtures were made and tested at 35°, as before:

A = 5 c.c. 7 per cent. NaCl sol. + 5 c.c. rennet sol.
B = 5 c.c. distilled water + 5 c.c. rennet sol.
C = 1 c.c. 7 per cent. NaCl sol. + 5 c.c. rennet sol.
D = 1 c.c. distilled water + 5 c.c. rennet sol.

Tested as follows:

	First Test	Second Test
1 c.c. *A* + 5 c.c. milk	—coagulated in 66 min.	coagulated in 67 min.
1 c.c. *B* + 5 c.c. milk	—coagulated in 50 min.	coagulated in 51 min.
1 c.c. *C* + 5 c.c. milk	—coagulated in 30 min.	coagulated in 32 min.
1 c.c. *D* + 5 c.c. milk	—coagulated in 30 min.	coagulated in 30 min.

It is thus seen that the retarding action of the evaporated filtrate in this experiment is due to the presence of seven per cent. NaCl.*

EXPERIMENT X.

Filtration through Chamberland filter.—The series of filtrations tabulated below show that the rennet molecule is also unable to pass through the pores of the Pasteur filter, size *F*. The bougie before use was thoroughly cleansed, ignited, and sterilized by autoclaving before each individual filtration. For each filtration at vacuum pressure 190 c.c. of a 1 : 10,000 rennet solution were employed. The filtrations at 50 pounds' pressure were made with 195 c.c. The filtrates were all tested for alkalinity and found to be neutral in reaction. The controls and tests, at 36°, were made as in the case of the Berkefeld filtrations.

TABLE XXIX.

No.	Pressure	Time of Filtration	Coagulating Action of		
			Control		Filtrate
			Milk	Rennet	
1.......	27½-27⅞ in.	3½ min.	Negative	Coag. in 25 min	Neg. in 10¼ hrs.
2.......	27⅞ in.	3⅓ min.	Negative	Coag. in 22 min.	Neg. in 9¼ hrs.
3.......	27½-28⅛ in.	4 min.	Negative	Coag. in 23 min.	Neg. in 7 hrs.
1.......	50 ℔s.	80 sec.	Negative	Coag. in 20 min.	Neg. in 13 hrs, 10 min.
2.......	50 ℔s.	70 sec.	Negative	Coag. in 20 min.	Neg. in 11 hrs. 30 min.
3.......	50 ℔s.	75 sec.	Negative	Coag. in 14 min.	Neg. in 10 hrs.

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* *Cf.* OPPENHEIMER, *Die Fermente, etc.*, p. 149.

EXPERIMENT XI.

Filtration through collodion sacs.—Filtration through collodion sacs (as previously described) resulted in the absolute retention of the enzyme by the filter.

The filtrates and controls were tested at 36° as in previous experiments.

TABLE XXX.

NO.	DILUTION	PRESSURE	TIME OF FILTRATION	AM'T FILTERED	COAGULATING ACTION OF		
					Control		Filtrate
					Milk	Rennet	
1.......	1:10,000	2-3 in.	2 hrs.	6½ c.c.	Negative	Coag. in 23 min.	Neg. in 6 hrs.
2.......	1:1,000	2½ in.	2½ hrs.	4 c.c.	Negative	Coag. in 2 min.	Neg. in 8½ hrs.
3.......	1:1,000	4 in.	1 hr.	4 c.c.	Negative	Coag. in 3 min.	Neg. in 9 hrs.

EXPERIMENT XII.

Dialysis through collodion sacs.— It will be seen from the following, which is one of several similar experiments, that rennet dialyzes readily through the collodion sac.

Seven very thin sacs were prepared by rolling the tube used in their preparation twice in thin collodion. These were designated *C*, 1, 2, 3, 4, 5, and 6. Five cubic centimeters of a 1:1,000 rennet solution, previously boiled for five minutes, were placed in the sac marked *C*, and served as a control. In each of sacs 1–6, inclusive, were placed 5 c.c. of a fresh 1:1,000 solution. Each sac was then immersed in a test-tube on foot containing 25 c.c. of milk and placed in a nincubator at 36°. The first evidence that the enzyme dialyzed through the walls of the sac was seen in the formation of a coagulum which adhered in clumps to the outside of the sac. This "bushing" increased in extent until in time the entire contents of the tube coagulated. The results obtained were as follows:

Slight "bushing" in Nos. 1, 3, 5, and 6 in 6 hrs.
Marked "bushing" in Nos. 1, 2, 3, 4, and 5 in 9 hrs.
Slight "bushing" in No. 6 in 9 hrs.
No "bushing" in control in 9 hrs.

When next examined at the end of twenty-one hours, the control showed a soft, creamy coagulum extending through the mass of milk, whereas in Tubes 1–6, inclusive, the curd formed a solid layer at the bottom of the tube, while above it was a layer of clear liquid. Solid masses of curd were adherent to each sac.

The sacs employed in this experiment were tested before and after use, and were found to be free from leaks.

As in the case of ptyalin, dialysis was shown to occur through the same sacs, which did not allow filtration of the enzyme.

By way of contrast to the behavior of the collodion sac may be given an experiment wherein dialysis was attempted by placing within a Berkefeld bougie 10 c.c. of a 1:10,000 rennet. The bougie was placed in a test-tube in 40 c.c. of milk and set aside at 36°. No dialysis occurred; the result in this case agreeing with that of Lea and Dickinson (*vide supra*).

The foregoing study of the behavior of rennet shows (1) that it is removed from its solution by filtration through hardened filter paper; (2) that this removal is not due to fixation by the paper; (3) that it is readily destroyed by aeration and surface action; (4) that it is removed completely from its solution by the pores of a new Berkefeld bougie, even under high pressure; (5) that this removal is mechanical and is not due to chemical destruction; (6) that it is removed in like manner by the Chamberland filter; (7) that it readily dialyzes through the collodion sac, but not through a Berkefeld bougie; (8) that it is removed from its solution when filtered through a collodion sac.

PEPSIN.

PAPER FILTRATION.

The pepsin used in these experiments was that of Parke, Davis & Co. The solutions were prepared by dissolving the preparation in 0.7 per cent. NaCl. They were filtered before use, either before or after dilution, through a single thickness of ordinary filter paper. The action of filtrates, controls, etc., in these experiments was tested by adding 0.1 g. of fibrin to 5 c.c. of the pepsin solution, to which had been added 1 c.c. of a 10 per cent. HCl. This fibrin was obtained from hog's blood and was well washed first in running water, then in distilled water, and was carefully pressed, just before weighing, between several thicknesses of filter paper, in order to remove as much moisture as possible.

EXPERIMENT I.

The following table gives the results of three sets of filtrations of a 1:16,000 pepsin solution. A common control was used for the "6×2" and "8×2" filtrates. The arrangement of filters, it may be added, was the same as that heretofore employed.

TABLE XXXI.

No.	Duration of Filtration	Digestion Temperature	Time Required to Produce Solution of Fibrin by						
			"2×2"		"4×2"		"6×2"		"8×2"
			Control	Filtrate	Control	Filtrate	Control	Filtrate	Filtrate
1...	2 hrs.	37°	5 hrs.	5 hrs.+	3-4 hrs.	4-5 hrs.	3 hrs.	Neg. in 24 hrs.	Neg. in 24 hrs.
2...	2 hrs. 35 min.	38°	2-3 hrs.	3 hrs.+	2-3 hrs.	¾ dissol. in 18 hrs.	2-3 hrs.	Neg. in 18 hrs.	Neg. in 18 hrs.
3...	1 hr. 50 min.	35°	¾ dissol. in 4 hrs.	4 hrs.	⅘ dissol. in 4 hrs.	6–7 hrs.	¾ dissol. in 3 hrs.	¾ dissol. in 11 hrs.	¾ dissol. in 11 hrs.

A blank control was made in each case with 0.1 g. fibrin in 5 c.c. of 0.7 per cent. NaCl containing 0.1 c.c. of a 10 per cent. HCl. These were uniformly negative during the time of the experiment.

This experiment clearly shows that pepsin is removed from its solution by hardened filter paper.

To determine if this decrease in the activity of the enzyme solutions was due to fixation by the filter paper, experiments were performed similar to those demonstrating the fixation of ptyalin, but no fixation could be demonstrated. Aeration was observed to have a destructive effect upon the enzyme, and this effect was aided by the surface action of the paper bits or garnets over which the pepsin was aerated.

EXPERIMENT II.

The effect of Berkefeld filters.— The table below shows the effect of Berkefeld filtration on 1:16,000 pepsin solutions. One hundred and ninety cubic centimeters were filtered in each instance. The filtrate and control tests and blank controls were made as before. The digestion was carried on at 36–37°. The bougie was sterilized before each filtration by autoclaving.

TABLE XXXII.

No.	Pressure	Time of Filtration	Time Required to Produce Solution of Fibrin by		
			Control		Filtrate
			Blank	Pepsin	
1........	27⅞ in.	65 sec.	Negative	1–2 hrs.	10–20 hrs.
2........	27¾ in.	65 sec.	Negative	*ca.* 1½ hrs.	8–18 hrs.
3........	27¾ in.	65 sec.	Negative	*ca.* 1½ hrs.	½ dissol. in 10 hrs.
4........	50 ℔s.	16 sec.	Negative	1½–2 hrs.	12 hrs. +
5........	50 ℔s.	16 sec.	Negative	*ca.* 2 hrs.	¾ dissol. in 18 hrs.
6........	50 ℔s.	20 sec.	Negative	*ca.* 2 hrs.	¾ dissol. in 18 hrs.
7........	100 ℔s.	5 sec.	Negative	*ca.* 2 hrs.	¾ dissol. in 24 hrs. +
8........	100 ℔s.	10 sec.	Negative	*ca.* 2 hrs.	¾ dissol. in 24 hrs.
9........	100 ℔s.	8 sec.	Negative	2–3 hrs.	¾ dissol. in 24 hrs.

In Tests 1-4, inclusive, hog fibrin several days old was used.

The two following filtrations were made with the same bougie used above. The tests were carried out at 36°.

TABLE XXXIII.

No.	Pressure	Time of Filtration	Solvent Action on Fibrin of		
			Control		Filtrate
			Blank	Pepsin	
1........	28 in.	70 sec.	Neg.	Solution in 2-3 hrs.	Negative in 15 hrs.
2........	27⅞ in.	70 sec.	Neg.	Solution in 2-3 hrs.	Negative in 14 hrs.

In Table XXXII a less ready passage is observed at 100 pounds' pressure than at vacuum pressure. The subsequent filtrations through the same bougie at vacuum pressure indicated a clogging of the pores of the filter by pepsin and other substances in the successive filtrations. Fermi and Pernossi (*loc. cit.*) call attention to the fact that the passage of pepsin through the Chamberland bougie is less ready when the bougie used had previously been employed for the filtration of colloidal substances.

These experiments go to show that a marked difference exists between rennet and pepsin in regard to the passage through the Berkefeld filter.

EXPERIMENT III.

Filtration through collodion sac.—In a series of trials it was shown that pepsin does not pass through the collodion filter. The results are given in the following table. The pepsin solutions employed were 1:16,000 and the digestion was carried on at 37°.

TABLE XXXIV.

No.	Pressure	Am't Filtered	Time of Filtration	Time Required to Dissolve Fibrin by		
				Control		Filtrate
				Blank	Pepsin	
1.......	2½-3 in.	5 c.c.	45 min.	Partial 24-38 hrs.	*ca.* 2 hrs.	Neg. in 38 hrs.
2.......	1-3 in.	....	1½ hrs.	Neg. in 34 hrs.	2 hrs.	Neg. in 34 hrs.
3.......	2¼ in. (*ca.*)	8 c.c.	30 min.	Neg. in 48 hrs.	2-3 hrs.	Sol. in 24 hrs.
4.......	2 in. (*ca.*)	6 c.c.	19 min.	Neg. in 2¼ hrs.	*ca.* 2 hrs.	Sol. in 2¼ hrs.
5.......	1-2½ in.	4 c.c.	2¼ hrs.	Neg. in 24 hrs.	*ca.* 2 hrs.	Neg. in 24 hrs.
6.......	1-3 in.	4 c.c.	2¼ hrs.	Neg. in 24 hrs.	*ca.* 2 hrs.	Neg. in 24 hrs.

The rapidity of the filtration process in trials Nos. 3 and 4 made it quite probable that some leakage of the sacs occurred. With the exception of these two, the results indicate that pepsin cannot pass through the collodion filter.

EXPERIMENT IV.

Dialysis through collodion sacs.—As with other enzymes heretofore stated it will be seen that although pepsin does not filter through the collodion wall, it is dialyzable through that membrane. The following experiment will illustrate the dialysis of pepsin:

Seven thin collodion sacs were prepared as before described and were labeled *S*, *C*, *C'*, 1, 2, 3, 4, and 5, respectively. Each sac was immersed in a test-tube on foot containing 20 c.c. of a 0.7 per cent. NaCl, acidulated with 0.4 c.c. of a 10 per cent. HCl, and 0.1 g. fibrin. Each of the sacs *C* and *C'* contained 5 c.c. of a 1:5,000 pepsin solution, previously boiled for five minutes, and served as controls. In each of sacs 1–5, inclusive, were placed 5 c.c. of a freshly prepared 1:5,000 pepsin solution. All the tubes were set aside in an incubator at 37°.

When observed at the end of twenty-eight hours, the solution of the fibrin was complete in Tube 4. A small sediment remained in Nos. 1, 2, and 5. A small lump of undissolved fibrin remained in No. 3. The fibrin placed in this tube consisted of a solid piece, and not of shreds as in the other tubes. The controls showed no solution. At end of forty-eight hours the solution of the fibrin in sacs 1–5, inclusive, was practically complete, whereas in the control tubes there remained a comparatively large sediment.

The sacs were tested at the close of the experiment, and were found free from leaks.

The foregoing details show (1) that pepsin is removed from dilute solution by filtration through hardened filtered paper; (2) that this removal is probably not due to fixation by the paper; (3) that aeration and surface action are injurious to pepsin; (4) that pepsin is removed from solution by the Berkefeld bougie and by the collodion filter; (5) and that it readily dialyzes through the collodion sac.

PANCREATIN.

The pancreatin used in these experiments was the commercial preparation of Parke, Davis & Co. The solutions employed, except when otherwise indicated, were of a strength of 1 : 500 in 0.7 per cent. NaCl. Before use they were filtered through a single thickness of ordinary filter paper.

The pancreatin was tested by adding to 5 c.c. of the solution 0.08 c.c. of a 10 per cent. Na_2CO_3, and 0.1 g. fibrin. One hundred and ninety cubic centimeters were filtered in each instance. Control tests were made with the unfiltered solution. Blank control consisted of 0.1 g. fibrin in 5 c.c. of a 0.7 per cent. NaCl containing 0.08 c.c. of a 10 per cent. Na_2CO_3.

EXPERIMENT I.

Filtration through paper.—The following table gives the results obtained on filtering the pancreatin solution through a series of hardened filter paper. The tests were made at a temperature of 36°. A common control served for the "6 × 2" and "8 × 2" filtrates. Blank controls, it may be added, were negative in every trial.

TABLE XXXV.

No.	Duration of Filtration	Time Required to Dissolve Fibrin by						
		"2 × 2"		"4 × 2"		"6 × 2"		"8 × 2"
		Control	Filtrate	Control	Filtrate	Control	Filtrate	Filtrate
1	3 hrs. 15 min.	8-9 hrs.	*ca.* 10 hrs	8-9 hrs.	10-18 hrs.	8-9 hrs.	31-42 hrs.	Neg., 18 hrs
2	2 hrs. 15 min.	7-10 hrs.	7-10 hrs.	*ca.* 10 hrs	10-21 hrs.	9 hrs.	22-23 hrs.	Neg., 48 hrs
3	2 hrs. 20 min.	7½-9 hrs.	*ca.* 9 hrs.	7-8 hrs.	8-21 hrs.	6-7¾ hrs.	21-24 hrs.	Sol. in 48-72 hrs.

It is evident from these tests that pancreatin is removed from its solution by hardened filter paper.

Subsequent aeration experiments failed to demonstrate the fixation of enzyme. Aeration was found to have a destructive effect on the enzyme, which destructive effect was increased by the surface action of paper and of garnets.

EXPERIMENT II.

Filtration through the Berkefeld filter.—The results obtained by Fermi and Pernossi and others, in connection with the filtration of pancreatin through porous filters have already been noted. Below are tabulated the results of a series of filtrations through the Berkefeld bougie. The filtrates and controls were tested in the manner described above. One hundred and ninety cubic centimeters of the 1:500 enzyme solution were filtered in each instance. The digestion temperature was 36-37°.

TABLE XXXVI.

No.	Pressure	Time of Filtration	Time Required to Produce Solution of Fibrin by		
			Control		Filtrate
			Blank	Pancreatin	
1........	22⅜ in.	40 sec.	Negative	5 hrs.	24-25 hrs.
2........	28 3/16 in.	55 sec.	Negative	7 + hrs.	24-30 hrs.
3........	28 in.	75 sec.	Negative	8 hrs.	¾ diss. in 48 hrs.
4........	50 lbs.	16 sec.	Negative	9½-11½ hrs.	24 hrs.
5........	50 lbs.	16 sec.	Negative	8¼-10¼ hrs.	35 hrs.
6........	100 lbs.	About 12 sec.	Negative	8 hrs.	50 hrs.
7.......	100 lbs.	About 10 sec.	Negative	8 hrs.	30-45 hrs.
8........	100 lbs.	About 10 sec.	Negative	8 hrs.	30-45 hrs.

It will be observed that. as in the case of pepsin, the bougie becomes clogged by successive filtration.

After the second filtration at 50 pounds' pressure, the bougie in use was accidentally broken. The subsequent filtrations at 100 pounds were then performed with a new bougie. During the process of filtration the tube connecting the filtering apparatus with the pressure tank broke, after 98 c.c. of the liquid had passed through. The succeeding portion of liquid which filtered through at gravity pressure was collected separately. The time of filtration at 50 pounds' pressure was seven or eight seconds. The gravity filtration which followed gave 3-4 c.c. per minute. The filtrates and controls were tested as before with the following results:

Blank control — negative in 48 hrs.
Pancreatin control — solution in 10-21 hrs.
Gravity filtrate — negative in 48 hours.
50 pounds' filtrate — negative in 48 hrs.

Evidences of bacterial growth in these tubes appeared at the end of forty-eight hours, and hence the observations were discontinued. There was in this instance no passage of pancreatin through the bougie. This same bougie was used in 100-pound filtrations tabulated above.

EXPERIMENT III.

Filtration through collodion sacs.—The collodion filter is impermeable to trypsin as the following table will show.

TABLE XXXVII.

No.	Pressure	Time of Filtration	Amount Filtered	Temp. of Digestion	Time Required to Dissolve Fibrin by		
					Control		Filtrate
					Blank	Pancreatin	
1.......	1½-3 in.	2 hrs.	5 c.c.	36°	Neg.	6½-8 hrs.	Neg. in 48 hrs.
2.......	2-3 1/16 in.	2 hrs.	5½ c.c.	37°	Neg.	7 + hrs.	Neg. in 72 hrs.
3.......	2-2½ in.	1½ hrs.	5½ c.c.	37-38°	Neg.	*ca.* 8 hrs.	Neg. in 72 hrs.

Dialysis through collodion sacs.—Unlike the enzymes already studied, pancreatin does not dialyze through the collodion sac. The dialysis experiments were performed exactly as in the case of preceding enzymes. The tubes were kept under observation in one instance as long as eleven days, but at no time was there any evidence of dialysis.

Inasmuch as the commercial pancreatin was employed for the preceding experiments, it was deemed desirable to check these with a freshly prepared trypsin solution. Accordingly, a fresh beef pancreas was ground up in a sausage machine, and the pulp was extracted with water. The filtered solution was precipitated with alcohol, and the precipitate was then digested with glycerin. The glycerin extract thus obtained was found to possess a marked proteolytic action. All attempts to dialyze this trypsin solution or to filter through the collodion sac gave negative results, as did the commercial preparation.

These experiments show (1) that pancreatin is removed from its solution by filtration through hardened filter paper; (2) that this removal evidently is not due to fixation; (3) that the enzyme is injuriously affected by aeration and by surface action; (4) that it passes in varying degree through the Berkefeld bougie, in one instance being totally retained; (5) that it does not filter through the collodion sac; (6) and that, unlike all the other enzymes experimented upon, it does not dialyze through the collodion sac.

CONCLUSIONS.

The results obtained in this study may be advantageously and briefly summarized as follows:

I. The removal from solution by paper filtration of ptyalin, rennet, pepsin, and pancreatin.

II. The fixation of ptyalin by hardened filter paper.

III. The injurious effect of aeration and of surface action on taka diastase, rennet, pepsin, and pancreatin.

IV. The passage of ptyalin and taka diastase through the Berkefeld bougie; the absolute retention of rennet, even under high pressure, by the Berkefeld bougie; the partial retention of pepsin; and the partial retention (in one case total retention) of pancreatin.

V. The total retention of rennet by the Chamberland bougie.

VI. The total retention of ptyalin, rennet, pepsin, and pancreatin by the collodion filter, and the partial passage through this filter of taka diastase.

VII. The dialysis of ptyalin, taka diastase, rennet, and pepsin, but not of pancreatin, through the collodion membrane.

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REFERENCES.

1. MUSCULUS, *Compt. rend. Ac. Sc.*, 1874, 78, p. 132; 1876, 82, p. 333.
2. LEA, *Jour. of Physiol.*, 6, p. 136.
3. MIQUEL, *Fermentation ammoniacale et ferments de l'urée* (Paris: Carré et Naud, 1898), p. 262.
4. WORTMANN, *Bot. Zeitung*, 1890, 48, pp. 581, 597, 617, 633, 657.
5. BROWN AND MORRIS, *Jour. Chem. Soc.*, 1893, 63, p. 634.
6. BROWN AND HERRON, *Proc. Roy. Soc.*, 1880, 30, p. 399.
7. VINES, *Ann. of Bot.*, 1891, 5, p. 410.
8. KASTLE AND LOEVENHART, *Amer. Chem. Jour.*, 1900, 24, p. 498.
9. SZUMOWSKI, *Arch. de Physiol.*, 5^e Série, 1898, 10, p. 160.
10. EFFRONT, *Les enzymes et leur applications* (Paris: Carré et Naud, 1899), p. 21.
11. CHEVREUL, *Compt. rend. Ac. Sc.*, 1853, 36, p. 986.
12. GORSLINE, *Contributions to Medical Research* (Dedicated to Dr. V. C. Vaughan; Ann Arbor, Mich.: Wahr, 1903), p. 390.
13. RODET AND GUÉCHOFF, *Compt. rend. Soc. Biol.*, 1900, 52, pp. 965–67.
14. CRENDIROUPOULIS AND RUFFER, *ibid.*, pp. 1109, 1110.
15. NOVY, *Laboratory Work in Bacteriology* (Ann Arbor: Wahr, 1899), p. 499.
16. GAUTIER, *Compt. rend. Ac. Sc.*, 1882, 94, p. 652.
17. FERMI AND PERNOSSI, *Ztschr. f. Hyg.*, 1894, 18, p. 106.
18. ACHALME, *Ann. de l'Inst. Past.*, 1901, 15, p. 739.
19. FERMI. See Fermi and Pernossi.
20. BUCHNER, *Die Zymasegärung* (Edward Buchner, Hans Buchner, and Martin Hahn; München and Berlin: R. Oldenbourg, 1903).
21. FERNBACH, *Ann. de l'Inst. Past.*, 1890, 4, p. 641.
22. EFFRONT, *Enzymes and Their Applications* (Translated by S. C. Prescott; New York: Wiley & Sons, 1902), p. 57.
23. EHRLICH AND MORGENROTH, *Berl. klin. Wchnschr.*, 1900, 37, p. 682.

24. VEDDER, *Jour. Med. Research*, 1903, 9, pp. 475–501.
25. OPPENHEIMER, *Die Fermente und ihre Wirkungen* (Leipzig : Vogel, 1900), p. 37.
26. LEA, *Proc. Roy. Soc.*, 1883, 36, p. 55.
27. LEA AND DICKINSON, *Jour. of Physiol.*, 1890, 11, p. 310.
28. ROBERTS, *On the Digestive Ferments*, etc. (London: Smith, Elder & Co., 1880), p. 67.
29. CONN, *Centralbl. f. Bakt.*, 1892, 12, p. 223.
30. KORSCHUN, *Ztschr. f. Physiol. Chem.*, 1903, 37, p. 366.
31. NOVY, *Loc. cit.*, p. 471.
32. DUCLAUX, *Traité de microbiologie* (Paris: Masson et C^{ie}, 1900), 2, p. 271.